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In Re Application of:

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For: METHODS FOR GENERATING GENETICALLY ALTERED ANTIBODY-  
PRODUCING CELL LINES WITH IMPROVED ANTIBODY  
CHARACTERISTICS

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Box ☒ Sequence  
☐ Provisional ☐ Design

Assistant Commissioner for Patents  
Washington DC 20231

Sir:

PATENT APPLICATION TRANSMITTAL LETTER

Transmitted herewith for filing, please find

☒ A Utility Patent Application under 37 C.F.R. 1.53(b).

It is a continuing application, as follows:

☐ continuation ☐ divisional ☐ continuation-in-part of prior application number  
\_\_\_\_/\_\_\_\_.

☐ A Provisional Patent Application under 37 C.F.R. 1.53(c).

☐ A Design Patent Application (submitted in duplicate).

Including the following:

- ☐ Provisional Application Cover Sheet.
- ☒ New or Revised Specification, including pages 1 to 41 containing:
- ☒ Specification
  - ☒ Claims
  - ☒ Abstract
  - ☐ Substitute Specification, including Claims and Abstract.
- ☐ The present application is a continuation application of Application No. \_\_\_\_\_ filed \_\_\_\_\_. The present application includes the Specification of the parent application which has been revised in accordance with the amendments filed in the parent application. Since none of those amendments incorporate new matter into the parent application, the present revised Specification also does not include new matter.
- ☐ The present application is a continuation application of Application No. \_\_\_\_\_ filed \_\_\_\_\_, which in turn is a continuation-in-part of Application No. \_\_\_\_\_ filed \_\_\_\_\_. The present application includes the Specification of the parent application which has been revised in accordance with the amendments filed in the parent application. Although the amendments in the parent C-I-P application may have incorporated new matter, since those are the only revisions included in the present application, the present application includes no new matter in relation to the parent application.
- ☐ A copy of earlier application Serial No. \_\_\_\_\_ Filed \_\_\_\_\_, including Specification, Claims and Abstract (pages 1 - @@), to which no new matter has been added TOGETHER WITH a copy of the executed oath or declaration for such earlier application and all drawings and appendices. Such earlier application is hereby incorporated into the present application by reference.
- ☐ Please enter the following amendment to the Specification under the Cross-Reference to Related Applications section (or create such a section) : "This Application:
- ☐ is a continuation of ☐ is a divisional of ☐ claims benefit of U.S. provisional Application Serial No. \_\_\_\_\_ filed \_\_\_\_\_

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- ☐ Signed Statement attached deleting inventor(s) named in the prior application.
- ☐ A Preliminary Amendment.
- ☒ 7 Sheets of ☒ Formal ☐ Informal Drawings.
- ☐ Petition to Accept Photographic Drawings.
- ☐ Petition Fee
- ☒ An ☐ Executed ☒ Unexecuted Declaration or Oath and Power of Attorney.
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- ☐ The prior application is assigned of record to \_\_\_\_\_
- ☐ Priority is claimed under 35 U.S.C. § 119 of Patent Application No. \_\_\_\_\_  
filed \_\_\_\_\_ in \_\_\_\_\_ (country).
- ☐ A Certified Copy of each of the above applications for which priority is  
claimed:
- ☐ is enclosed.
- ☐ has been filed in prior application Serial No. \_\_\_\_\_ filed \_\_\_\_\_.
- ☐ An ☐ Executed or ☐ Copy of Executed Earlier Statement Claiming Small Entity  
Status under 37 C.F.R. 1.9 and 1.27
- ☐ is enclosed.
- ☐ has been filed in prior application Serial No. \_\_\_\_\_ filed \_\_\_\_\_,  
said status is still proper and desired in present case.

- ☒ Diskette Containing DNA/Amino Acid Sequence Information.
- ☒ Statement to Support Submission of DNA/Amino Acid Sequence Information.
- ☐ The computer readable form in this application \_\_\_\_\_, is identical with that filed in Application Serial Number \_\_\_\_\_, filed \_\_\_\_\_. In accordance with 37 CFR 1.821(e), please use the ☐ first-filed, ☐ last-filed or ☐ only computer readable form filed in that application as the computer readable form for the instant application. It is understood that the Patent and Trademark Office will make the necessary change in application number and filing date for the computer readable form that will be used for the instant application. A paper copy of the Sequence Listing is ☐ included in the originally-filed specification of the instant application, ☐ included in a separately filed preliminary amendment for incorporation into the specification.
- ☐ Information Disclosure Statement.
- ☐ Attached Form 1449.
- ☐ Copies of each of the references listed on the attached Form PTO-1449 are enclosed herewith.
- ☐ A copy of Petition for Extension of Time as filed in the prior case.
- ☐ Appended Material as follows: \_\_\_\_\_.
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- ☐ Other as follows: \_\_\_\_\_  
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**FEE CALCULATION:**

- ☐ Cancel in this application original claims \_\_\_\_\_ of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

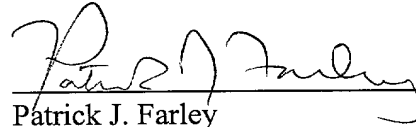
				SMALL ENTITY		NOT SMALL ENTITY	
				RATE	FEE	RATE	FEE
PROVISIONAL APPLICATION				\$75.00	\$	\$150.00	\$
DESIGN APPLICATION				\$160.00	\$	\$320.00	\$
UTILITY APPLICATIONS BASE FEE				\$355.00	\$	\$710.00	\$710.00
UTILITY APPLICATION; ALL CLAIMS CALCULATED AFTER ENTRY OF ALL AMENDMENTS							
	No. Filed	No. Extra					
TOTAL CLAIMS	72 - 20 =	52		\$9 each	\$	\$18 each	\$936.00
INDEP. CLAIMS	7 - 3 =	4		\$40 each	\$	\$80 each	\$320.00
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				\$135	\$	\$270	\$
ADDITIONAL FILING FEE					\$		\$
TOTAL FILING FEE DUE					\$		\$1,966.00

- ☒ A Check is enclosed in the amount of \$ 1,966.00 .
- ☒ The Commissioner is authorized to charge payment of the following fees and to refund any overpayment associated with this communication or during the pendency of this application to deposit account 23-3050. This sheet is provided in duplicate.
- ☐ The foregoing amount due.
- ☒ Any additional filing fees required, including fees for the presentation of extra claims under 37 C.F.R. 1.16.
- ☒ Any additional patent application processing fees under 37 C.F.R. 1.17 or 1.20(d).
- ☐ The issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance.
- ☒ The Commissioner is hereby requested to grant an extension of time for the appropriate length of time, should one be necessary, in connection with this filing or any future filing submitted to the U.S. Patent and Trademark Office in the above-

identified application during the pendency of this application. The Commissioner is further authorized to charge any fees related to any such extension of time to deposit account 23-3050. This sheet is provided in duplicate.

**SHOULD ANY DEFICIENCIES APPEAR** with respect to this application, including deficiencies in payment of fees, missing parts of the application or otherwise, the United States Patent and Trademark Office is respectfully requested to promptly notify the undersigned.

Date: 11/7/00

  
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## METHODS FOR GENERATING GENETICALLY ALTERED ANTIBODY- PRODUCING CELL LINES WITH IMPROVED ANTIBODY CHARACTERISTICS

### TECHNICAL FIELD OF THE INVENTION

5           The invention is related to the area of antibody maturation and cellular production. In particular, it is related to the field of mutagenesis.

### BACKGROUND OF THE INVENTION

10           The use of antibodies to block the activity of foreign and/or endogenous polypeptides provides an effective and selective strategy for treating the underlying cause of disease. In particular is the use of monoclonal antibodies (MAb) as effective therapeutics such as the FDA approved ReoPro (Glaser, V. (1996) Can ReoPro repolish tarnished monoclonal therapeutics? *Nat. Biotechnol.* 14:1216-1217), an anti-platelet MAb from Centocor; Herceptin (Weiner, L.M. (1999) Monoclonal antibody therapy of cancer. *Semin. Oncol.* 26:43-51), an anti-Her2/neu MAb from Genentech; and Synagis (Saez-Llorens, X.E., *et al.* (1998) Safety and pharmacokinetics of an intramuscular humanized monoclonal antibody to respiratory syncytial virus in premature infants and infants with bronchopulmonary dysplasia. *Pediat. Infect. Dis. J.* 17:787-791), an anti-respiratory syncytial virus MAb produced by Medimmune.

20           Standard methods for generating MAbs against candidate protein targets are known by those skilled in the art. Briefly, rodents such as mice or rats are injected with a purified antigen in the presence of adjuvant to generate an immune response (Shield, C.F., *et al.* (1996) A cost-effective analysis of OKT3 induction therapy in cadaveric kidney

transplantation. *Am. J. Kidney Dis.* 27:855-864). Rodents with positive immune sera are sacrificed and splenocytes are isolated. Isolated splenocytes are fused to melanomas to produce immortalized cell lines that are then screened for antibody production. Positive lines are isolated and characterized for antibody production. The direct use of rodent MAbs as human therapeutic agents were confounded by the fact that human anti-rodent antibody (HARA) responses occurred in a significant number of patients treated with the rodent-derived antibody (Khazaeli, M.B., *et al.*, (1994) Human immune response to monoclonal antibodies. *J. Immunother.* 15:42-52). In order to circumvent the problem of HARA, the grafting of the complementarity determining regions (CDRs), which are the critical motifs found within the heavy and light chain variable regions of the immunoglobulin (Ig) subunits making up the antigen binding domain, onto a human antibody backbone found these chimeric molecules are able to retain their binding activity to antigen while lacking the HARA response (Emery, S.C., and Harris, W.J. "Strategies for humanizing antibodies" In: ANTIBODY ENGINEERING C.A.K. Borrebaeck (Ed.) Oxford University Press, N.Y. 1995. pp. 159-183. A common problem that exists during the "humanization" of rodent-derived MAbs (referred to hereon as HAb) is the loss of binding affinity due to conformational changes in the 3 dimensional structure of the CDR domain upon grafting onto the human Ig backbone (U.S. Patent No. 5,530,101 to Queen *et al.*). To overcome this problem, additional HAb vectors are usually needed to be engineered by inserting or deleting additional amino acid residues within the framework region and/or within the CDR coding region itself in order to recreate high affinity HAbs (U.S. Patent No. 5,530,101 to Queen *et al.*). This process is a very time consuming procedure that involves the use of expensive computer modeling programs to predict changes that may lead to a high affinity HAb. In some instances the affinity of the HAb is never restored to that of the MAb, rendering them of little therapeutic use.

Another problem that exists in antibody engineering is the generation of stable, high yielding producer cell lines that is required for manufacturing of the molecule for clinical materials. Several strategies have been adopted in standard practice by those skilled in the art to circumvent this problem. One method is the use of Chinese Hamster Ovary (CHO) cells transfected with exogenous Ig fusion genes containing the grafted human light and heavy



chains to produce whole antibodies or single chain antibodies, which are a chimeric molecule containing both light and heavy chains that form an antigen-binding polypeptide (Reff, M.E. (1993) High-level production of recombinant immunoglobulins in mammalian cells. *Curr. Opin. Biotechnol.* 4:573-576). Another method employs the use of human lymphocytes  
5 derived from transgenic mice containing a human grafted immune system or transgenic mice containing a human Ig gene repertoire. Yet another method employs the use of monkeys to produce primate MAbs, which have been reported to lack a human anti-monkey response (Neuberger, M., and Gruggermann, M. (1997) Monoclonal antibodies. Mice perform a human repertoire. *Nature* 386:25-26). In all cases, the generation of a cell line that is capable  
10 of generating sufficient amounts of high affinity antibody poses a major limitation for producing sufficient materials for clinical studies. Because of these limitations, the utility of other recombinant systems such as plants are currently being explored as systems that will lead to the stable, high-level production of humanized antibodies (Fiedler, U., and Conrad, U. (1995) High-level production and long-term storage of engineered antibodies in transgenic  
15 tobacco seeds. *Bio/Technology* 13:1090-1093).

A method for generating diverse antibody sequences within the variable domain that results in HAbs and MAbs with high binding affinities to antigens would be useful for the creation of more potent therapeutic and diagnostic reagents respectively. Moreover, the generation of randomly altered nucleotide and polypeptide residues throughout an entire  
20 antibody molecule will result in new reagents that are less antigenic and/or have beneficial pharmacokinetic properties. The invention described herein is directed to the use of random genetic mutation throughout an antibody structure *in vivo* by blocking the endogenous mismatch repair (MMR) activity of a host cell producing immunoglobulins that encode biochemically active antibodies. The invention also relates to methods for repeated *in vivo*  
25 genetic alterations and selection for antibodies with enhanced binding and pharmacokinetic profiles.

In addition, the ability to develop genetically altered host cells that are capable of secreting increased amounts of antibody will also provide a valuable method for creating cell hosts for product development. The invention described herein is directed to the creation of  
30 genetically altered cell hosts with increased antibody production via the blockade of MMR.

The invention facilitates the generation of high affinity antibodies and the production of cell lines with elevated levels of antibody production. Other advantages of the present invention are described in the examples and figures described herein.

## 5 SUMMARY OF THE INVENTION

The invention provides methods for generating genetically altered antibodies (including single chain molecules) and antibody producing cell hosts *in vitro* and *in vivo*, whereby the antibody possess a desired biochemical property(s), such as, but not limited to, increased antigen binding, increased gene expression, and/or enhanced extracellular secretion  
10 by the cell host. One method for identifying antibodies with increased binding activity or cells with increased antibody production is through the screening of MMR defective antibody producing cell clones that produce molecules with enhanced binding properties or clones that have been genetically altered to produce enhanced amounts of antibody product.

The antibody producing cells suitable for use in the invention include, but are not  
15 limited to rodent, primate, or human hybridomas or lymphoblastoids; mammalian cells transfected and expressing exogenous Ig subunits or chimeric single chain molecules; plant cells, yeast or bacteria transfected and expressing exogenous Ig subunits or chimeric single chain molecules.

Thus, the invention provides methods for making hypermutable antibody-producing cells by introducing a polynucleotide comprising a dominant negative allele of a mismatch repair gene into cells that are capable of producing antibodies. The cells that are capable of producing antibodies include cells that naturally produce antibodies, and cells that are engineered to produce antibodies through the introduction of immunoglobulin encoding sequences. Conveniently, the introduction of polynucleotide sequences into cells is accomplished by transfection.

The invention also provides methods of making hypermutable antibody producing cells by introducing a dominant negative mismatch repair (MMR) gene such as *PMS2* (preferably human *PMS2*), *MLH1*, *PMS1*, *MSH2*, or *MSH2* into cells that are capable of producing antibodies. The dominant negative allele of a mismatch repair gene may be a truncation mutation of a mismatch repair gene (preferably a truncation mutation at codon 134,

or a thymidine at nucleotide 424 of wild-type *PMS2*). The invention also provides methods in which mismatch repair gene activity is suppressed. This may be accomplished, for example, using antisense molecules directed against the mismatch repair gene or transcripts.

Other embodiments of the invention provide methods for making a hypermutable antibody producing cells by introducing a polynucleotide comprising a dominant negative allele of a mismatch repair gene into fertilized eggs of animals. These methods may also include subsequently implanting the eggs into pseudo-pregnant females whereby the fertilized eggs develop into a mature transgenic animal. The mismatch repair genes may include, for example, *PMS2* (preferably human *PMS2*), *MLH1*, *PMS1*, *MSH2*, or *MSH2*. The dominant negative allele of a mismatch repair gene may be a truncation mutation of a mismatch repair gene (preferably a truncation mutation at codon 134, or a thymidine at nucleotide 424 of wild-type *PMS2*).

The invention further provides homogeneous compositions of cultured, hypermutable, mammalian cells that are capable of producing antibodies and contain a dominant negative allele of a mismatch repair gene. The mismatch repair genes may include, for example, *PMS2* (preferably human *PMS2*), *MLH1*, *PMS1*, *MSH2*, or *MSH2*. The dominant negative allele of a mismatch repair gene may be a truncation mutation of a mismatch repair gene (preferably a truncation mutation at codon 134, or a thymidine at nucleotide 424 of wild-type *PMS2*). The cells of the culture may contain *PMS2*, (preferably human *PMS2*), *MLH1*, or *PMS1*; or express a human *mutL* homolog, or the first 133 amino acids of hPMS2.

The invention further provides methods for generating a mutation in an immunoglobulin gene of interest by culturing an immunoglobulin producing cell selected for an immunoglobulin of interest wherein the cell contains a dominant negative allele of a mismatch repair gene. The properties of the immunoglobulin produced from the cells can be assayed to ascertain whether the immunoglobulin gene harbors a mutation. The assay may be directed to analyzing a polynucleotide encoding the immunoglobulin, or may be directed to the immunoglobulin polypeptide itself.

The invention also provides methods for generating a mutation in a gene affecting antibody production in an antibody-producing cell by culturing the cell expressing a dominant negative allele of a mismatch repair gene, and testing the cell to determine whether the cell

harbors mutations within the gene of interest, such that a new biochemical feature (*e.g.*, over-expression and/or secretion of immunoglobulin products) is generated. The testing may include analysis of the steady state expression of the immunoglobulin gene of interest, and/or analysis of the amount of secreted protein encoded by the immunoglobulin gene of interest. The invention also embraces prokaryotic and eukaryotic transgenic cells made by this process, including cells from rodents, non-human primates and humans.

Other aspects of the invention encompass methods of reversibly altering the hypermutability of an antibody producing cell, in which an inducible vector containing a dominant negative allele of a mismatch repair gene operably linked to an inducible promoter is introduced into an antibody-producing cell. The cell is treated with an inducing agent to express the dominant negative mismatch repair gene (which can be *PMS2* (preferably human *PMS2*), *MLH1*, or *PMS1*). Alternatively, the cell may be induced to express a human *mutL* homolog or the first 133 amino acids of hPMS2. In another embodiment, the cells may be rendered capable of producing antibodies by co-transfecting a preselected immunoglobulin gene of interest. The immunoglobulin genes of the hypermutable cells, or the proteins produced by these methods may be analyzed for desired properties, and induction may be stopped such that the genetic stability of the host cell is restored.

The invention also embraces methods of producing genetically altered antibodies by transfecting a polynucleotide encoding an immunoglobulin protein into a cell containing a dominant negative mismatch repair gene (either naturally or in which the dominant negative mismatch repair gene was introduced into the cell), culturing the cell to allow the immunoglobulin gene to become mutated and produce a mutant immunoglobulin, screening for a desirable property of said mutant immunoglobulin protein, isolating the polynucleotide molecule encoding the selected mutant immunoglobulin possessing the desired property, and transfecting said mutant polynucleotide into a genetically stable cell, such that the mutant antibody is consistently produced without further genetic alteration. The dominant negative mismatch repair gene may be *PMS2* (preferably human *PMS2*), *MLH1*, or *PMS1*. Alternatively, the cell may express a human *mutL* homolog or the first 133 amino acids of hPMS2.

The invention further provides methods for generating genetically altered cell lines that express enhanced amounts of an antigen binding polypeptide. These antigen-binding polypeptides may be, for example, immunoglobulins. The methods of the invention also include methods for generating genetically altered cell lines that secrete enhanced amounts of an antigen binding polypeptide. The cell lines are rendered hypermutable by dominant negative mismatch repair genes that provide an enhanced rate of genetic hypermutation in a cell producing antigen-binding polypeptides such as antibodies. Such cells include, but are not limited to hybridomas. Expression of enhanced amounts of antigen binding polypeptides may be through enhanced transcription or translation of the polynucleotides encoding the antigen binding polypeptides, or through the enhanced secretion of the antigen binding polypeptides, for example.

Methods are also provided for creating genetically altered antibodies *in vivo* by blocking the MMR activity of the cell host, or by transfecting genes encoding for immunoglobulin in a MMR defective cell host.

Antibodies with increased binding properties to an antigen due to genetic changes within the variable domain are provided in methods of the invention that block endogenous MMR of the cell host. Antibodies with increased binding properties to an antigen due to genetic changes within the CDR regions within the light and/or heavy chains are also provided in methods of the invention that block endogenous MMR of the cell host.

The invention provides methods of creating genetically altered antibodies in MMR defective Ab producer cell lines with enhanced pharmacokinetic properties in host organisms including but not limited to rodents, primates, and man.

These and other aspects of the invention are provided by one or more of the embodiments described below. In one embodiment of the invention, a method for making an antibody producing cell line hypermutable is provided. A polynucleotide encoding a dominant negative allele of a MMR gene is introduced into an antibody-producing cell. The cell becomes hypermutable as a result of the introduction of the gene.

In another embodiment of the invention, a method is provided for introducing a mutation into an endogenous gene encoding for an immunoglobulin polypeptide or a single chain antibody. A polynucleotide encoding a dominant negative allele of a MMR gene is

introduced into a cell. The cell becomes hypermutable as a result of the introduction and expression of the MMR gene allele. The cell further comprises an immunoglobulin gene of interest. The cell is grown and tested to determine whether the gene encoding for an immunoglobulin or a single chain antibody of interest harbors a mutation. In another aspect of the invention, the gene encoding the mutated immunoglobulin polypeptide or single chain antibody may be isolated and expressed in a genetically stable cell. In a preferred embodiment, the mutated antibody is screened for at least one desirable property such as, but not limited to, enhanced binding characteristics.

In another embodiment of the invention, a gene or set of genes encoding for Ig light and heavy chains or a combination therein are introduced into a mammalian cell host that is MMR defective. The cell is grown, and clones are analyzed for antibodies with enhanced binding characteristics.

In another embodiment of the invention, a method will be provided for producing new phenotypes of a cell. A polynucleotide encoding a dominant negative allele of a MMR gene is introduced into a cell. The cell becomes hypermutable as a result of the introduction of the gene. The cell is grown. The cell is tested for the expression of new phenotypes where the phenotype is enhanced secretion of a polypeptide.

These and other embodiments of the invention provide the art with methods that can generate enhanced mutability in cells and animals as well as providing cells and animals harboring potentially useful mutations for the large-scale production of high affinity antibodies with beneficial pharmacokinetic profiles.

## BRIEF DESCRIPTION OF THE DRAWINGS

**Figure 1.** Hybridoma cells stably expressing PMS2 and PMS134 MMR genes.

Shown is steady state mRNA expression of MMR genes transfected into a murine hybridoma cell line. Stable expression was found after 3 months of continuous growth. The (-) lanes represent negative controls where no reverse transcriptase was added, and the (+) lanes represent samples reverse transcribed and PCR amplified for the MMR genes and an internal housekeeping gene as a control.

**Figure 2.** Creation of genetically hypermutable hybridoma cells. Dominant negative

MMR gene alleles were expressed in cells expressing a MMR-sensitive reporter gene. Dominant negative alleles such as PMS134 and the expression of MMR genes from other species results in antibody producer cells with a hypermutable phenotype that can be used to produce genetically altered immunoglobulin genes with enhanced biochemical features as well as lines with increased Ig expression and/or secretion. Values shown represent the amount of converted CPRG substrate which is reflective of the amount of function  $\beta$ -galactosidase contained within the cell from genetic alterations within the pCAR-OF reporter gene. Higher amounts of  $\beta$ -galactosidase activity reflect a higher mutation rate due to defective MMR.

**Figure 3.** Screening method for identifying antibody-producing cells containing antibodies with increased binding activity and/or increased expression/secretion

**Figure 4.** Generation of a genetically altered antibody with an increased binding activity. Shown are ELISA values from 96-well plates, screened for antibodies specific to hIgE. Two clones with a high binding value were found in HB134 cultures.

**Figure 5.** Sequence alteration within variable chain of an antibody (a mutation within the light chain variable region in MMR-defective HB134 antibody producer cells). Arrows indicate the nucleotide at which a mutation occurred in a subset of cells from a clone derived from HB134 cells. Panel A: The change results in a Thr to Ser change within the light chain variable region. The coding sequence is in the antisense direction. Panel B: The change results in a Pro to His change within the light chain variable region.

**Figure 6.** Generation of MMR-defective clones with enhanced steady state Ig protein levels. A Western blot of heavy chain immunoglobulins from HB134 clones with high levels of MAb (>500ngs/ml) within the conditioned medium shows that a subset of clones express higher steady state levels of immunoglobulins (Ig). The H36 cell line was used as a control to measure steady state levels in the parental strain. Lane 1: fibroblast cells (negative control); Lane 2: H36 cell; Lane 3: HB134 clone with elevated MAb levels; Lane 4: HB134 clone with elevated MAb levels; Lane 5: HB134 clone with elevated MAb levels.

Methods have been discovered for developing hypermutable antibody-producing cells by taking advantage of the conserved mismatch repair (MMR) process of host cells.

Dominant negative alleles of such genes, when introduced into cells or transgenic animals,

increase the rate of spontaneous mutations by reducing the effectiveness of DNA repair and thereby render the cells or animals hypermutable. Hypermutable cells or animals can then be utilized to develop new mutations in a gene of interest. Blocking MMR in antibody-producing cells such as but not limited to: hybridomas; mammalian cells transfected with genes encoding for Ig light and heavy chains; mammalian cells transfected with genes encoding for single chain antibodies; eukaryotic cells transfected with Ig genes, can enhance the rate of mutation within these cells leading to clones that have enhanced antibody production and/or cells containing genetically altered antibodies with enhanced biochemical properties such as increased antigen binding. The process of MMR, also called mismatch proofreading, is carried out by protein complexes in cells ranging from bacteria to mammalian cells. A MMR gene is a gene that encodes for one of the proteins of such a mismatch repair complex. Although not wanting to be bound by any particular theory of mechanism of action, a MMR complex is believed to detect distortions of the DNA helix resulting from non-complementary pairing of nucleotide bases. The non-complementary base on the newer DNA strand is excised, and the excised base is replaced with the appropriate base, which is complementary to the older DNA strand. In this way, cells eliminate many mutations that occur as a result of mistakes in DNA replication.

Dominant negative alleles cause a MMR defective phenotype even in the presence of a wild-type allele in the same cell. An example of a dominant negative allele of a MMR gene is the human gene *hPMS2-134*, which carries a truncating mutation at codon 134 (SEQ ID NO:15). The mutation causes the product of this gene to abnormally terminate at the position of the 134th amino acid, resulting in a shortened polypeptide containing the N-terminal 133 amino acids. Such a mutation causes an increase in the rate of mutations, which accumulate in cells after DNA replication. Expression of a dominant negative allele of a mismatch repair gene results in impairment of mismatch repair activity, even in the presence of the wild-type allele. Any allele which produces such effect can be used in this invention. Dominant negative alleles of a MMR gene can be obtained from the cells of humans, animals, yeast, bacteria, or other organisms. Such alleles can be identified by screening cells for defective MMR activity. Cells from animals or humans with cancer can be screened for defective mismatch repair. Cells from colon cancer patients may be particularly useful. Genomic



DNA, cDNA, or mRNA from any cell encoding a MMR protein can be analyzed for variations from the wild type sequence. Dominant negative alleles of a MMR gene can also be created artificially, for example, by producing variants of the *hPMS2-134* allele or other MMR genes. Various techniques of site-directed mutagenesis can be used. The suitability of such alleles, whether natural or artificial, for use in generating hypermutable cells or animals can be evaluated by testing the mismatch repair activity caused by the allele in the presence of one or more wild-type alleles, to determine if it is a dominant negative allele.

A cell or an animal into which a dominant negative allele of a mismatch repair gene has been introduced will become hypermutable. This means that the spontaneous mutation rate of such cells or animals is elevated compared to cells or animals without such alleles. The degree of elevation of the spontaneous mutation rate can be at least 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, 100-fold, 200-fold, 500-fold, or 1000-fold that of the normal cell or animal. The use of chemical mutagens such as but limited to methane sulfonate, dimethyl sulfonate, O6-methyl benzadine, MNU, ENU, etc. can be used in MMR defective cells to increase the rates an additional 10 to 100 fold that of the MMR deficiency itself.

According to one aspect of the invention, a polynucleotide encoding for a dominant negative form of a MMR protein is introduced into a cell. The gene can be any dominant negative allele encoding a protein, which is part of a MMR complex, for example, *PMS2*, *PMS1*, *MLH1*, or *MSH2*. The dominant negative allele can be naturally occurring or made in the laboratory. The polynucleotide can be in the form of genomic DNA, cDNA, RNA, or a chemically synthesized polynucleotide.

The polynucleotide can be cloned into an expression vector containing a constitutively active promoter segment (such as but not limited to CMV, SV40, Elongation Factor or LTR sequences) or to inducible promoter sequences such as the steroid inducible pIND vector (Invitrogen), where the expression of the dominant negative MMR gene can be regulated. The polynucleotide can be introduced into the cell by transfection.

According to another aspect of the invention, an immunoglobulin (Ig) gene, a set of Ig genes or a chimeric gene containing whole or parts of an Ig gene can be transfected into MMR deficient cell hosts, the cell is grown and screened for clones containing genetically altered Ig genes with new biochemical features. MMR defective cells may be of human,

primates, mammals, rodent, plant, yeast or of the prokaryotic kingdom. The mutated gene encoding the Ig with new biochemical features may be isolated from the respective clones and introduced into genetically stable cells (*i.e.*, cells with normal MMR) to provide clones that consistently produce Ig with the new biochemical features. The method of isolating the

5 Ig gene encoding Ig with new biochemical features may be any method known in the art. Introduction of the isolated polynucleotide encoding the Ig with new biochemical features may also be performed using any method known in the art, including, but not limited to transfection of an expression vector containing the polynucleotide encoding the Ig with new biochemical features. As an alternative to transfecting an Ig gene, a set of Ig genes or a  
10 chimeric gene containing whole or parts of an Ig gene into an MMR deficient host cell, such Ig genes may be transfected simultaneously with a gene encoding a dominant negative mismatch repair gene into a genetically stable cell to render the cell hypermutable.

Transfection is any process whereby a polynucleotide is introduced into a cell. The process of transfection can be carried out in a living animal, *e.g.*, using a vector for gene  
15 therapy, or it can be carried out *in vitro*, *e.g.*, using a suspension of one or more isolated cells in culture. The cell can be any type of eukaryotic cell, including, for example, cells isolated from humans or other primates, mammals or other vertebrates, invertebrates, and single celled organisms such as protozoa, yeast, or bacteria.

In general, transfection will be carried out using a suspension of cells, or a single cell,  
20 but other methods can also be applied as long as a sufficient fraction of the treated cells or tissue incorporates the polynucleotide so as to allow transfected cells to be grown and utilized. The protein product of the polynucleotide may be transiently or stably expressed in the cell. Techniques for transfection are well known. Available techniques for introducing polynucleotides include but are not limited to electroporation, transduction, cell fusion, the  
25 use of calcium chloride, and packaging of the polynucleotide together with lipid for fusion with the cells of interest. Once a cell has been transfected with the MMR gene, the cell can be grown and reproduced in culture. If the transfection is stable, such that the gene is expressed at a consistent level for many cell generations, then a cell line results.

An isolated cell is a cell obtained from a tissue of humans or animals by mechanically  
30 separating out individual cells and transferring them to a suitable cell culture medium, either

with or without pretreatment of the tissue with enzymes, *e.g.*, collagenase or trypsin. Such isolated cells are typically cultured in the absence of other types of cells. Cells selected for the introduction of a dominant negative allele of a mismatch repair gene may be derived from a eukaryotic organism in the form of a primary cell culture or an immortalized cell line, or  
5 may be derived from suspensions of single-celled organisms.

A polynucleotide encoding for a dominant negative form of a MMR protein can be introduced into the genome of an animal by producing a transgenic animal. The animal can be any species for which suitable techniques are available to produce transgenic animals. For example, transgenic animals can be prepared from domestic livestock, *e.g.*, bovine, swine,  
10 sheep, goats, horses, etc.; from animals used for the production of recombinant proteins, *e.g.*, bovine, swine, or goats that express a recombinant polypeptide in their milk; or experimental animals for research or product testing, *e.g.*, mice, rats, guinea pigs, hamsters, rabbits, etc. Cell lines that are determined to be MMR defective can then be used as a source for producing genetically altered immunoglobulin genes *in vitro* by introducing whole, intact  
15 immunoglobulin genes and/or chimeric genes encoding for single chain antibodies into MMR defective cells from any tissue of the MMR defective animal.

Once a transfected cell line or a colony of transgenic animals has been produced, it can be used to generate new mutations in one or more gene(s) of interest. A gene of interest can be any gene naturally possessed by the cell line or transgenic animal or introduced into  
20 the cell line or transgenic animal. An advantage of using such cells or animals to induce mutations is that the cell or animal need not be exposed to mutagenic chemicals or radiation, which may have secondary harmful effects, both on the object of the exposure and on the workers. However, chemical mutagens may be used in combination with MMR deficiency, which renders such mutagens less toxic due to an undetermined mechanism. Hypermutable  
25 animals can then be bred and selected for those producing genetically variable B-cells that may be isolated and cloned to identify new cell lines that are useful for producing genetically variable cells. Once a new trait is identified, the dominant negative MMR gene allele can be removed by directly knocking out the allele by technologies used by those skilled in the art or by breeding to mates lacking the dominant negative allele to select for offspring with a  
30 desired trait and a stable genome. Another alternative is to use a CRE-LOX expression

system, whereby the dominant negative allele is spliced from the animal genome once an animal containing a genetically diverse immunoglobulin profile has been established. Yet another alternative is the use of inducible vectors such as the steroid induced pIND (Invitrogen) or pMAM (Clonetech) vectors which express exogenous genes in the presence of corticosteroids.

Mutations can be detected by analyzing for alterations in the genotype of the cells or animals, for example by examining the sequence of genomic DNA, cDNA, messenger RNA, or amino acids associated with the gene of interest. Mutations can also be detected by screening for the production of antibody titers. A mutant polypeptide can be detected by identifying alterations in electrophoretic mobility, spectroscopic properties, or other physical or structural characteristics of a protein encoded by a mutant gene. One can also screen for altered function of the protein *in situ*, in isolated form, or in model systems. One can screen for alteration of any property of the cell or animal associated with the function of the gene of interest, such as but not limited to Ig secretion.

Examples of mismatch repair proteins and nucleic acid sequences include the following:

#### PMS2 (mouse) (SEQ ID NO:5)

MEQTEGVSTE	CAKAIKPIDG	KSVHQICSGQ	VILSLSTAVK	ELIENSVDAG	ATTIDLRLKD	60
YGVDLIEVSD	NGCGVEEENF	EGLALKHHTS	KIQEFADLTQ	VETFGFRGEA	LSSLICALSDV	120
TISTCHGSAS	VGTRLVFDHN	GKITQKTPYP	RPKGTTVSQ	HLFYTLPVRY	KEFQRNIKKE	180
YSKMVQVLQA	YCIISAGVRV	SCTNQLGQ GK	RHAVVCTSGT	SGMKENIGSV	FGQKQLQSLI	240
PFVQLPPSDA	VCEEYGLSTS	GRHKTFSTFR	ASFHSARTAP	GGVQQTGSFS	SSIRGPVTQQ	300
RSLSLSMRFY	HMYNRHQYPF	VVLNVSDVSE	CVDINVTDPK	RQILLQEEKL	LLAVLKTSLI	360
GMFDS DANKL	NVNQQPLLDV	EGNLVKLHTA	ELEKPVPGKQ	DNPSPLKSTA	DEKRVASISR	420
LREAFSLHPT	KEIKSRGPET	AELTRSFPE	KRGVLSSYPS	DVISYRGLRG	SQDKLVSPD	480
SPGDCMDREK	IEKDSGLSST	SAGSEEEFST	PEVASSFSSD	YNVSLED RP	SQETINCGDL	540
DCRPPGTGQS	LKPEDHGYQC	KALPLARLSP	TNAKRFKTEE	RPSNVNISQR	LPGPQSTSAA	600
EVDVAIKMNK	RIVLLEFSLS	SLAKRMKQLQ	HLKAQNKHEL	SYRKFRAKIC	PGENQAAEDE	660
LRKEISKSMF	AEMEILGQFN	LGFIIVTKLKE	DLFLVDQHAA	DEKYNFEMLQ	QHTVLQAQRL	720
ITPQTLNLTA	VNEAVLIENL	EIFRKNGFDF	VIDEDAPVTE	RAKLISLPTS	KNWTFGPQDI	780
DELIFMLSDS	PGVMCRPSRV	RQMFASRACR	KSVMIGTALN	ASEMKKLITH	MGEMDHPWNC	840
PHGRPTMRHV	ANLDVISQN					859

#### PMS2 (mouse cDNA) (SEQ ID NO:6)

gaattccggt	gaaggtcctg	aagaatttcc	agattcctga	gtatcattgg	aggagacaga	60
taacctgtcg	tcaggtaacg	atgggtgtata	tgcaacagaa	atgggtgttc	ctggagacgc	120
gtcttttccc	gagagcggca	ccgcaactct	cccgcggtga	ctgtgactgg	aggagtcctg	180
catccatgga	gcaaaccgaa	ggcgtgagta	cagaatgtgc	taaggccatc	aagcctattg	240
atgggaagtc	agtcocatcaa	attgtttctg	ggcaggtgat	actcagttta	agcaccgctg	300
tgaaggagtt	gatagaaaaat	agtgtagatg	ctggtgctac	tactattgat	ctaaggctta	360
aagactatgg	ggtggacctc	attgaagttt	cagacaatgg	atgtggggta	gaagaagaaa	420

5 actttgaagg tctagctctg aaacatcaca catctaagat tcaagagttt gccgacctca 480  
 cgcagggttg aacttttcggc ttctcgggggg aagctctgag ctctctgtgt gcactaagtg 540  
 atgtcactat atctacctgc cacgggtctg caagcgttgg gactcgactg gtgtttgacc 600  
 ataattgggaa aatcacccag aaaactccct acccccgacc taaaggaacc acagtcagt 660  
 tgcagcactt atttttatata ctaccctgtg gttacaaaga gtttcagagg aacattaaaa 720  
 aggagtattc caaaatgggt caggtcttac aggcgtactg tatcatctca gcaggcgtcc 780  
 gtgtaagctg cactaatcag ctgggacagg ggaagcggca cgctgtggtg tgcacaagcg 840  
 gcacgtctgg catgaaggaa aatatcggtt ctgtgtttgg ccagaagcag ttgcaaagcc 900  
 10 tcatctcttt tgttcagctg ccccttagtg acgctgtgtg tgaagagtac ggctgagca 960  
 cttcaggacg ccacaaaacc ttttctacgt ttctgggcttc atttcacagt gcacgcacgg 1020  
 cgccgggagg agtgaacag acaggcagtt ttctctcatc aatcagaggc cctgtgacc 1080  
 agcaaagggtc tctaagcttg tcaatgaggt tttatcacat gtataaccgg catcagtacc 1140  
 catttgctgt ccttaacgtt tccgttgact cagaatgtgt ggatattaat gtaactccag 1200  
 ataaaaggca aattctacta caagaagaga agctattgct ggccgtttta aagacctcct 1260  
 15 tgataggaat gtttgacagt gatgcaaaca agcttaatgt caaccagcag cactgctag 1320  
 atgttggaagg taacttagta aagctgcata ctgcagaact agaaaagcct gtgccaggaa 1380  
 agcaagataa ctctccttca ctgaagagca cagcagacga gaaaaggta gcatccatct 1440  
 ccaggctgag agaggccttt tctcttcata ctactaaaga gatcaagtct aggggtccag 1500  
 20 agactgctga actgacacgg agttttccaa gtgagaaaag gggcgtgtta tctctttatc 1560  
 cttcagacgt catctcttac agaggcctcc gtggctcgca ggacaaattg gtgagtccca 1620  
 cggacagccc tgggtgactgt atggacagag agaaaataga aaaagactca gggctcagca 1680  
 gcacctcagc tggctctgag gaagagttca gcacccaga agtggccagt agcttttagca 1740  
 gtgactataa cgtgagctcc ctgaagaca gaccttctca ggaaaccata aactgtggtg 1800  
 25 acctggactg ccgtcctcca ggtacaggac ttactctgaa gccagaagac catggatata 1860  
 aatgcaaagc tctacctcta gctcgtctgt caccacaaa tgccaagcgc ttcaagacag 1920  
 aggaaagacc ctcaaagtgc aacatttctc aaagattgcc tggctcctag agcacctcag 1980  
 cagctgaggt cgatgtagcc ataaaaatga ataagagaat cgtgctcctc gagtctctct 2040  
 tgagttctct agctaagcga atgaagcagt tacagcacct aaaggcgcag aacaaacatg 2100  
 aactgagtta cagaaaaatt agggccaaga ttctccctgg agaaaaccac gcagcagaag 2160  
 30 atgaactcag aaaagagatt agtaaatcga tgtttgcaga gatggagatc ttgggtcagt 2220  
 ttaacctggg atttatagta accaaactga aagaggacct cttcctgggt gaccagcatg 2280  
 ctgcgatga gaagtacaac tttgagatgc tgcagcagca cacggtgctc caggcgaga 2340  
 ggctcatcac accccagact ctgaacttaa ctgctgtcaa tgaagctgta ctgatagaaa 2400  
 atctggaaat attcagaaaag aatggctttg actttgtcat tgatgaggat gctccagtca 2460  
 35 ctgaaagggc taaattgatt tccttaccac ctagtaaaaa ctggaccttt ggaccacaag 2520  
 atatatagta actgatcttt atgttaagtg acagccctgg ggtcatgtgc cggccctcac 2580  
 gagtcagaca gatgtttgct tccagagcct gtcggaagtc agtgatgatt ggaacggcgc 2640  
 tcaatgcgag cgagatgaag aagctcatca cccacatggg tgagatggac caccctgga 2700  
 actgccccca cggcaggcca accatgaggg acgttgccaa tctggatgct atctctcaga 2760  
 40 actgacacac cccttgtagc atagagttta ttacagattg ttcggtttgc aaagagaagg 2820  
 ttttaagtaa tctgattatc gttgtacaaa aattagcatg ctgctttaat gtactggatc 2880  
 catttaaaaag cagtgttaag gcaggcatga tggagtgttc ctctagctca gctacttggg 2940  
 tgatccggtg ggagctcatg tgagcccagg actttgagac cactccgagc cacattcatg 3000  
 agactcaatt caaggacaaa aaaaaaaga tatttttgaa gccttttaaa aaaaaa 3056

## PMS2 (human) (SEQ ID NO:7)

50 MERAESSSTE PAKAIKPIDR KSVHQICSGQ VVLSLSTAVK ELVENS LDAG ATNIDLKLDK 60  
 YGVDLIEVSD NGCGVEEENF EGLTLKHHTS KIQEFADLTQ VETFGFRGEA LSSLCALSDV 120  
 TISTCHASAK VGTRLMFDHN GKIIQKTPYP RPRGTTVSQ QLFSTLPVRH KEFQRNIKKE 180  
 YAKMVQVLHA YCIISAGIRV SCTNLQGGK RQPVVCTGGS PSIKENIGSV FGQKQLQSLI 240  
 PFVQLPPSDS VCEEYGLSCS DALHNLFYIS GFISQCTHGV GRSSTRQFF FINRRPCDPA 300  
 KVCRLVNEVY HMYNRHQYPF VVLNISVDSE CVDINVTDPK RQILLQEEKL LLAVLKTSLI 360  
 GMFSDVNKL NVSQQLLDV EGNLIKMHAA DLEKPMVEKQ DQSPSLRTGE EKKDVSISRL 420  
 55 REAFSLRHTT ENKPHSPKTP EPRRSPLGQK RGMSSSTSG AISDKGVL RP QKEAVSSSHG 480  
 PSDPTDRAEV EKDSGHGST VDSGFSIPD TSHCSSEYA ASSPGDRGSQ EHVDSEKAP 540  
 ETDDSFSDVD CHSNQEDTGC KFRVLPQPTN LATPNTKRFK KEEILSSSDI CQKLVNTQDM 600  
 SASQVDVAVK INKKVPLDF SMSSLAKRIK QLHHEAQOSE GEQNYRKFRA KICPGENQAA 660  
 EDELKKEISK TMFAEMELIG QFNLFITITK LNEDIFIVDQ HATDEKYNFE MLQQHTVLQG 720  
 60 QRLIAPQTLN LTAVNEAVLI ENLEIFRKNG FDFVIDENAP VTERAKLISL PTSKNWTFGP 780  
 QDVDELIFML SDSPGVMC RP SRVKQMFASR ACRKSVMIGT ALNTSEMKKL ITHMGEMDHP 840  
 WNCPHGRPTM RHIANLGVIS QN 862

## PMS2 (human cDNA) (SEQ ID NO:8)

5 cgaggcggat cgggtgttgc atccatggag cgagctgaga gctcgagtag agaacctgct 60  
 aaggccatca aacctattga tcggaagtca gtccatcaga tttgctctgg gcaggtaggta 120  
 ctgagtctaa gcactgcggt aaaggagtta gtagaaaaca gtctggatgc tggtagccact 180  
 aatattgata taaagcttaa ggactatgga gtggatctta ttgaagtttc agacaatgga 240  
 tgtggggtag aagaagaaaa cttcgaaggc ttaactctga aacatcacac atctaagatt 300  
 caagagtttg ccgacctaac tcagggttgaa acttttggct ttcgggggga agctctgagc 360  
 10 tcaactttgtg cactgagcga tgaccattt tctacctgcc acgcatcggc gaaggttgga 420  
 actcgactga tgtttgatca caatgggaaa attatccaga aaaccccta ccccgcccc 480  
 agagggaacca cagtcagcgt gcagcagtta ttttccacac tacctgtgcg ccataaggaa 540  
 tttcaaagga atattaagaa ggagtatgcc aaaatgggtcc aggtcttaca tgcatactgt 600  
 atcatttcag caggcatccg tgtaagttgc accaatcagc ttggacaagg aaaacgacag 660  
 cctgtggtat gcacaggtgg aagccccagc ataaaggaaa atatcggctc tgtgtttggg 720  
 15 cagaagcagt tgcaaaagcct cattcctttt gttcagctgc cccctagtga ctccgtgtgt 780  
 gaagagtacg gtttgagctg ttccgatgct ctgcataatc ttttttacct ctccaggtttc 840  
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 20 gatataatg ttactccaga taaaaggcaa attttgctac aagaggaaaa gcttttgttg 1080  
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 gaaaagccca tggtagaaaa gcaggatcaa tccccttcat taaggactgg agaagaaaaa 1260  
 25 aaagacgtgt ccatttccag actgcgagag gccttttctc ttcgtcacac aacagagaac 1320  
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 gactcggggc acggcagcac ttcctgtgat tctgaggggt tcagcatccc agacacgggc 1560  
 30 agtcactgca gcagcgagta tgccggccagc tcccagggg acaggggctc gcaggaacat 1620  
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 35 aagaaagttg tgcccttga cttttctatg agttctttag ctaaacgaat aaagcagtta 1920  
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 tgtcctggag aaaatcaagc agccgaagat gaactaagaa aagagataag taaaacgatg 2040  
 tttgcagaaa tggaaatcat tggtcagttt aacctgggat ttataataac caaactgaat 2100  
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 40 cagcagcaca ccgtgctcca ggggcagagg ctcatagcac ctcagactct caacttaact 2220  
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 agtaaaaaact ggaccttcgg accccaggac gtcgatgaac tgatcttcat gctgagcgac 2400  
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 45 cggaagtcgg tgatgattgg gactgtcctt aacacaagcg agatgaagaa actgatcacc 2520  
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 atgaaacctg ctacttaaaa aaaatacaca tcacacccat ttaaaagtga tcttgagaac 2760  
 50 cttttcaaac c 2771

## PMS1 (human) (SEQ ID NO:9)

55 MKQLPAATVR ILSQQIITS VVSVKELIE NSLDAGATSV DVKLENYGFD KIEVRDNGEG 60  
 IKAVDAPVMA MKYYTSKINS HEDLENLTTY GFRGEALGSI CCIAEVLITT RTAADNFSTQ 120  
 YVLDSGSHIL SQKPSHLGQG TTVTALRLFK NLPVRKQFYS TAKKCKDEIK KIQDLMSFG 180  
 ILKPDLRIVE VHNKAVIWQK SRVSDHKMAL MSVLGTAVMN NMESFQYHSE ESQIYLSGFL 240  
 PKCDADHSFT SLSTPERSFI FINSRPVHQK DILKLIRHHY NLKCLKESTR LYPVFFLKID 300  
 VPTADVVDNL TPDKSQVLLQ NKESVLIALE NLMTTCYGPL PSTNSYENNK TDVSAADIVL 360  
 SKTAETDVLV NKVESSGKNY SNVDTSVIPF QNDMHNDESG KNTDDCLNHQ ISIGDFGYGH 420  
 CSSEISNIDK NTKNAFQDIS MSNVSWENSQ TEYSKTCFIS SVKHTQSENG NKDHIDESGE 480  
 60 NEEEEAGLENS SEISADEWSR GNILKNSVGE NIEPVKILVP EKSLPCKVSN NNYPIPEQMN 540  
 LNEDSCNKKK NVIDNKSGKV TAYDLLSNRV IKKPMASAL FVQDHRPQFL IENPKTSLED 600

ATLQIEELWK	TLSEEEKLKY	EEKATKDLER	YNSQMKRAIE	QESQMSLKDG	RKKIKPTSAW	660
NLAQKHKLKT	SLSNQPKLDE	LLQSQIEKRR	SQNIKMVQIP	FSMKNLKINF	KKQNKVDLEE	720
KDEPCLIHNL	RFPDAWLMTS	KTEVMLLNPY	RVEEALLFKR	LLENHKLPAE	PLEKPIMLTE	780
SLFNGSHYLD	VLYKMTADDQ	RYSGSTYLS	PRLTANGFKI	KLIPGVSITE	NYLEIEGEMAN	840
CLPFYGVADL	KEILNAILNR	NAKEVYECRP	RKVISYLEGE	AVRLSRQLPM	YLSKEDIQDI	900
IYRMKHQFGN	EIKECVHGRP	FFHHLTYLPE	TT			932

## PMS1 (human) (SEQ ID NO:10)

10	ggcacgagtg	gctgcttgcg	gctagtggtg	ggtaattgcc	tgcctcgcg	tagcagcaag	60
	ctgctctgtt	aaaagcgaaa	atgaaacaat	tgcctgcggc	aacagttcga	ctcctttcaa	120
	gttctcagat	catcacttcg	gtggtcagtg	ttgtaaaaga	gcttattgaa	aactccttgg	180
	atgctggtgc	cacaagcgta	gatgttaaac	tggagaacta	tggatttgat	aaaattgagg	240
	tgcgagataa	cggggagggg	atcaaggctg	ttgatgcacc	tgtaatggca	atgaagtact	300
	acacctcaaa	aataaatagt	catgaagatc	ttgaaaattt	gacaacttac	ggttttcgtg	360
15	gagaagcctt	ggggtcaatt	tgttgtatag	ctgagggttt	aattacaaca	agaacggctg	420
	ctgataaatt	tagcaccag	tatgttttag	atggcagtg	ccacatactt	tctcagaaac	480
	cttcacatct	tgggtcaagg	acaactgtaa	ctgctttaag	attattttaag	aatctacctg	540
	taagaaagca	gttttactca	actgcaaaaa	aatgtaaaga	tgaaataaaa	aagatccaag	600
	atctcctcat	gagctttggg	atccttaaac	ctgacttaag	gattgtcttt	gtacataaca	660
20	aggcagttat	ttggcagaaa	agcagagtat	cagatcacaa	gatggctctc	atgtcagttc	720
	tggggactgc	tgttatgaac	aatatggaat	cctttcagta	ccactctgaa	gaatctcaga	780
	tttatctcag	tggatttctt	ccaaagtgtg	atgcagacca	ctctttcact	agtctttcaa	840
	caccagaaaag	aagtttcatc	ttcataaaca	gtcgaccagt	acatcaaaaa	gatatcttaa	900
	agttaatccg	acatcattac	aatctgaaat	gcctaaagga	atctactcgt	ttgtatcctg	960
25	ttttctttct	gaaaatcgat	gttctctacg	gtgatgttga	tgtaaattta	acaccagata	1020
	aaagccaagt	attattacaa	aataaggaat	ctgttttaat	tgctcttgaa	aatctgtatga	1080
	cgacttggtt	tggaccatta	cctagtacaa	attcttatga	aaataataaa	acagatgttt	1140
	ccgcagctga	catcgttctt	agtaaaacag	cagaaacaga	tgtgcttttt	aataaagtgg	1200
	aatcatctgg	aaagaattat	tcaaattgtt	atacttcagt	cattccattc	caaaatgata	1260
30	tgcataatga	tgaatctgga	aaaaacactg	atgattgttt	aaatcaccag	ataagtattg	1320
	gtgacttttg	ttatggtcat	tgtagttagt	aaattttctaa	cattgataaa	aacactaaga	1380
	atgcattttca	ggacattttca	atgagtaatg	tatcatggga	gaactctcag	acggaatata	1440
	gtaaaacttg	ttttataagt	tccgttaagc	acaccagtc	agaaaatggc	aataaagacc	1500
	atatagatga	gagtggggaa	aatgaggaag	aagcaggtct	tgaaaactct	tcgaaaattt	1560
35	ctgcagatga	gtggagcagg	ggaaatatac	ttaaaaaattc	agtgggagag	aatattgaac	1620
	ctgtgaaaat	tttagtgctt	gaaaaaagtt	taccatgtta	agtaagtaat	aataattatc	1680
	caatccctga	acaaatgaat	cttaatgaag	attcatgtta	caaaaaatca	aatgtaatag	1740
	ataataaatc	tggaaaagtt	acagottatg	atttacttag	caatcgagta	atcaagaaac	1800
	ccatgtcagc	aagtgtctct	tttgtttcaag	atcatcgctc	tcagtttctc	atagaaaaac	1860
40	ctaagactag	tttagaggat	gcaacactac	aaattgaaga	actgtggaag	acattgagtg	1920
	aagaggaaaa	actgaaatat	gaagagaagg	ctactaaaga	cttggaacga	tacaatagtc	1980
	aaatgaagag	agccattgaa	caggagtcac	aaatgtcact	aaaagatggc	agaaaaaaga	2040
	taaaacccac	cagcgcatgg	aatttggccc	agaagcacaa	gttaaaaaacc	tcattatcta	2100
	atcaacccaaa	acttgatgaa	ctccttcagt	cccaaattga	aaaaagaagg	agtcaaaaata	2160
45	ttaaaatggt	acagatcccc	ttttctatga	aaaacttaaa	aataaatttt	aagaaacaaa	2220
	acaaaagtga	cttagaagag	aaggatgaac	cttgcttgat	ccacaatctc	aggtttctctg	2280
	atgcatggct	aatgacatcc	aaaacagagg	taatgtttat	aaatccatat	agagtagaag	2340
	aagccctgct	atttaaaaga	cttcttgaga	atcataaact	tcctgcagag	ccactggaaa	2400
	agccaattat	gttaacagag	agtcttttta	atggatctca	ttatttagac	gttttatata	2460
50	aaatgacagc	agatgaccaa	agatacagtg	gatcaactta	cctgtctgat	cctcgtctta	2520
	cagcgaatgg	tttcaagata	aaattgatac	caggagtttc	aattactgaa	aattacttgg	2580
	aaatagaagg	aatggctaata	tgtctcccat	tctatggagt	agcagattta	aaagaaattc	2640
	ttaatgctat	attaaacaga	aatgcaaagg	aagttttatga	atgtagacct	cgaaaagtga	2700
	taagtatttt	agagggagaa	cgagtgcgtc	tatccagaca	attaccatg	tacttatcaa	2760
55	aagaggacat	ccaagacatt	atctacagaa	tgaagcacca	gttttgaaat	gaaattaaag	2820
	agtgtgttca	tgggtgcccc	ttttttcatc	atttaacctta	tcttcagaa	actacatgat	2880
	taaatatggt	taagaagatt	agttaaccatt	gaaattgggt	ctgtcataaa	acagcatgag	2940
	tctggtttta	aattatcttt	gtattatgtg	tcacatgggt	attttttaaa	tgaggattca	3000
	ctgacttggt	tttatattga	aaaaagttcc	acgtattgtta	gaaaacgtaa	ataaactaat	3060
60	aac						3063

## MSH2 (human) (SEQ ID NO:11)

5	MAVQPKETLQ	LESAAEVGFV	RFFQGMPEKP	TTTVRLFDRG	DFYTAHGEDA	LLAAREVFKT	60
	QGVIKYMGPA	GAKNLQSVVL	SKMNFESFVK	DLLLVRQYRV	EVYKNRAGNK	ASKENDWYLA	120
	YKASPGNLSQ	FEDILFGNND	MSASIGVVG	KMSAVDQGRQ	VGVGYSVDSIQ	RKLGLCEFPD	180
	NDQFSNLEAL	LIQIGPKCEV	LPGGETAGDM	GKLRQIIQRG	GILITERKKA	DFSTKDIYQD	240
	LNRLKGGKKG	EQMNSAVLPE	MENQVAVSSL	SAVIKFLELL	SDDSNFGQFE	LTTFDFSQYM	300
10	KLDIAAVRAL	NLFQGSVEDT	TGSQSLAALL	NKCKTPQGQR	LVNQWIKQPL	MDKNRIEERL	360
	NLVEAFVEDA	ELRQTLQEDL	LRRFPDLNRL	AKKFQQAAN	LQDCYRLYQG	INQLPNVIAQ	420
	LEKHEGKHQK	LLLAVFVTPL	TDLRSDFSKF	QEMIETTLDM	DQVENHEFLV	KPSFDPNLSE	480
	LREIMNDLEK	KMQSTLISAA	RDLGLDPGKQ	IKLDSSAQFG	YYFRVTCKEE	KVLRNNKNFS	540
	TVDIQKNGVK	FTNSKLTSLN	EEYTKNKTEY	EEAQDAIVKE	IVNISSGYVE	PMQTLNDVLA	600
15	QLDAVVSFAH	VSNQAPVPYV	RPAILEKGQG	RIILKASRHA	CVEVQDEIAF	IPNDVYFEKD	660
	KQMFHIIITGP	NMGGKSTYIR	QTGVIVLMAQ	IGCFVPCESA	EVSIVDCILA	RVGAGDSQLK	720
	GVSTFMAEML	ETASILRSAT	KDSLIIIDEL	GRGTSTYDGF	GLAWAISEYI	ATKIGAFCMF	780
	ATHFHELTAL	ANQIPTVNNL	HVTALTTEET	LTMLYQVKKG	VCDQSFQIHV	AELANFPKHV	840
	IECAKQKALE	LEEFQYIGES	QGYDIMEPAA	KKCYLEREQG	EKIIQEFLSK	VKQMPFTEMS	900
20	EENITIKLKQ	LKAEVIAKNN	SFVNEIISRI	KVTT			934

## MSH2 (human cDNA) (SEQ ID NO:12)

	ggcgggaaac	agcttagtg	gtgtggggtc	gcgcatcttc	ttcaaccagg	aggtagaggag	60
25	gtttcgacat	ggcgggtgcag	ccgaaggaga	cgctgcagtt	ggagagcgcg	gccgagggtcg	120
	gcttcgtgog	cttcttttcag	ggcatgccgg	agaagccgac	caccacagtg	cgctttttcg	180
	accggggcgga	cttctatacag	gogcacggcg	aggacgcgct	gctggccgcc	cgggaggtgt	240
	tcaagaccca	gggggtgatc	aagtacatgg	ggccggcagg	agcaaagaat	ctgcagagtg	300
	ttgtgcttag	taaaatgaat	tttgaatctt	ttgtaaaaga	tcttcttctg	gttcgtcagt	360
30	atagagttga	agtttataag	aatagagctg	gaaataaggc	atccaaggag	aatgattggt	420
	atttggcata	taaggcttct	cctggcaate	tctctcagtt	tgaagacatt	ctcttttggt	480
	acaatgatat	gtcagcttcc	attggtgttg	tgggtgttaa	aatgtccgca	gttgatggcc	540
	agagacaggt	tggagttggg	tatgtgtgatt	ccatacacag	gaaactagga	ctgtgtgaat	600
	tccctgataa	tgatcagttc	tccaatcttg	aggctctcct	catccagatt	ggaccaaagg	660
35	aatgtgtttt	acccggaggga	gagactgctg	gagacatggg	gaaactgaga	cagataattc	720
	aaagaggagg	aattctgatc	acagaaagaa	aaaaagctga	cttttccaca	aaagacattt	780
	atcaggacct	caaccggttg	ttgaaaggga	aaaagggaga	gcagatgaat	agtgtctgtat	840
	tgccagaaat	ggagaaatcag	gttgacgttt	catcactgtc	tgcggtaatc	aagtttttag	900
	aactcttatc	agatgattcc	aactttggac	agtttgaact	gactactttt	gacttcagcc	960
40	agtatatgaa	attggatatt	gcagcagtc	gagcccttaa	cttttttcag	ggttctgttg	1020
	aagataccac	tggctctcag	tctctggctg	ccttgctgaa	taagtgtaaa	acccctcaag	1080
	gacaaaagact	tggttaaccag	tggattaagc	agcctctcat	ggataagaac	agaatagagg	1140
	agagattgaa	tttagtgga	gctttttag	aagatgcaga	attgaggcag	actttacaag	1200
	aagattttact	tctgtcgattc	ccagatctta	accgacttgc	caagaagttt	caaagacaag	1260
45	cagcaaaactt	acaagattgt	taccgactct	atcagggtat	aaatcaacta	cctaattgta	1320
	tacaggctct	ggaaaaacat	gaaggaaaac	accagaaatt	attgttggca	gtttttgtga	1380
	ctoctcttac	tgatcttctg	tctgacttct	ccaagtttca	ggaaatgata	gaaacaactt	1440
	tagatatgga	tcagggtgga	aaccatgaat	tccttgtaaa	accttcattt	gatcctaata	1500
	tcagtgaatt	aaagaaaata	atgaatgact	tggaaaagaa	gatgcagtca	acattaataa	1560
	gtgcagccag	agatctttggc	ttggaccctg	gcaaacagat	taaactggat	tccagtgac	1620
50	agtttgata	ttactttctg	gtaacctgta	aggaagaaaa	agtccttctg	aacaataaaa	1680
	acttttagtac	tgtagatatc	cagaagaatg	gtgttaaatt	taccaacagc	aaattgactt	1740
	ctttaaatga	agagtatacc	aaaaataaaa	cagaatatga	agaagcccag	gatgccattg	1800
	ttaaagaaaat	tgtcaatatt	tcttcaggct	atgtagaacc	aatgcagaca	ctcaatgatg	1860
55	gttagctca	gctagatgct	gtgtcagct	tgtctacgt	gtcaaatgga	gcactgttct	1920
	catatgtacg	accagccatt	ttggagaaag	gacaaggaag	aattatatta	aaagcatcca	1980
	ggcatgcttg	tggtgaagtt	caagatgaaa	ttgcatttat	tcctaataac	gtatactttg	2040
	aaaaagataa	acagatgttc	cacatcatta	ctggccccaa	tatgggaggt	aaatcaacat	2100
	atattcgaca	aactggggtg	atagtactca	tggcccaaat	tgggtgtttt	gtgccatgtg	2160
	agtcagcaga	agtggtccatt	gtggactgca	tcttagcccg	agtaggggct	ggtgacagtc	2220



	aattgaaagg	agtctccacg	ttcatggctg	aaatgttgga	aactgcttct	atcctcaggt	2280
	ctgcaaccaa	agattcatta	ataatcatag	atgaattggg	aagaggaaact	tctacctacg	2340
	atggattttg	gtagcatgg	gctatatcag	aatacattgc	aacaaagatt	ggtgcttttt	2400
5	gcatgtttgc	aacccatttt	catgaactta	ctgccttggc	caatcagata	ccaactgtta	2460
	ataatctaca	tgtcacagca	ctcaccactg	aagagacctt	aactatgctt	tatcaggtga	2520
	agaaaagggt	ctgtgatcaa	agttttggga	ttcatgttgc	agagcttgct	aatttcccta	2580
	agcatgtaat	agagtgtgct	aaacagaaag	ccctggaact	tgaggagttt	cagtatatattg	2640
	gagaatcgca	aggatatgat	atcatggaac	cagcagcaaa	gaagtgctat	ctggaaaagag	2700
10	agcaagggtga	aaaaattatt	caggagttcc	tgtccaaggt	gaaacaaatg	ccctttactg	2760
	aaatgtcaga	agaaaacatc	acaataaagt	taaaacagct	aaaagctgaa	gtaatagcaa	2820
	agaataatag	ctttgtaaat	gaaatcattt	cacgaataaa	agttactacg	tgaaaaatcc	2880
	cagtaattgga	atgaaggtaa	tattgataag	ctattgtctg	taatagtttt	atattgtttt	2940
	atattaacccc	tttttccata	gtgttaactg	tcagtgccca	tgggctatca	acttaataag	3000
	atatttagta	atattttact	ttgaggacat	tttcaaagat	ttttattttg	aaaaatgaga	3060
15	gctgtaactg	aggactgttt	gcaattgaca	taggcaataa	taagtgatgt	gctgaatttt	3120
	ataaataaaa	tcatgtagtt	tgtgg				3145

## MLH1 (human) (SEQ ID NO:13)

20	MSFVAGVIRR	LDETVVNRIA	AGEVIQRPAN	AIKEMIENCL	DAKSTSIQVI	VKEGGLKLIQ	60
	IQDNGTGIRK	EDLDIVCERF	TTSKLQSFED	LASISTYGFR	GEALASISHV	AHVTITTKTA	120
	DGKCAYRASY	SDGKLKAPPK	PCAGNQGTQI	TVEDLFYNIA	TRRKALKNPS	EEYGKILEVV	180
	GRYSVHNAGI	SFSVKKQGET	VADVRTL PNA	STVDNIRSIF	GNAVSRELIE	IGCEDKTLAF	240
	KMNGYISNAN	YSVKKCIFLL	FINHRLVEST	SLRKAIETVY	AAYLPKNTHP	FLYLSLEISP	300
25	QNVDVNVHPT	KHEVHFLHEE	SILERVQQHI	ESKLLGSNSS	RMFTQTLLP	GLAGPSGEMV	360
	KSTTSLTSSS	TSGSSDKVYA	HQMVRTDSRE	QKLD AFLQPL	SKPLSSQPQA	IVTEDKTDIS	420
	SGRARQQDEE	MLELPAPAEV	AAKNQSLEGD	TTKGTSEMSE	KRGPTSSNPR	KRHRESDSVE	480
	MVEDDSRKEM	TAACP RRRI	INLTSVLSLQ	EEINEQGHEV	LREMLHNHSF	VGCVNPQWAL	540
	AQHQT KLYLL	NTTKLSEELF	YQILIYDFAN	FGVLR LSEPA	PLFDLAMLAL	DSPESGWTEE	600
30	DGPKEGLAEY	IVEFLKKKAE	MLADYFSLEI	DEEGNLIGLP	LLIDNYVPPL	EGLPIFILRL	660
	ATEVNWDEEK	ECFESLSKEC	AMFY SIRQY	ISEESTLSGQ	QSEVPGSIPN	SWKWTVEHIV	720
	YKALRSHILP	PKHFTEDGNI	LQLANLPDLY	KVFERC			756

## MLH1 (human) (SEQ ID NO:14)

35	cttggctctt	ctggcgccaa	aatgtogttc	gtggcagggg	ttattcgggc	gctggacgag	60
	acagtgggtga	accgcatcgc	ggcgggggaa	ggtatccagc	ggccagctaa	tgctatcaaa	120
	gagatgattg	agaactgttt	agatgcaaaa	tccacaagta	ttcaagtgat	tgtaaagag	180
	ggaggcctga	agttgattca	gatccaagac	aattggcaccg	ggatcaggaa	agaagactctg	240
	gatatgtgat	gtgaaagggt	cactactagt	aaactgcagt	cctttgagga	tttagccagt	300
	atttctacct	atggctttcg	aggtgaggct	ttggccagca	taagccatgt	ggctcatgtt	360
40	actattacaa	cgaaaacagc	tgatggaaag	tgtgcataca	gagcaagta	ctcagatgga	420
	aaactgaaag	cccctcctaa	accatgtgct	ggcaatcaag	ggacccagat	cacggtggag	480
	gacctttttt	acaacatagc	cacgaggaga	aaagctttta	aaaatccaag	tgaagaatat	540
	gggaaaaattt	tggaggttgt	tggcaggtat	tcagtacaca	atgcaggcat	tagttttctca	600
	gttaaaaaaac	aaggagagac	agtagctgat	gttaggcacac	tacccaatgc	ctcaaccgtg	660
45	gacaatatct	gctccatctt	tggaaatgct	gttagtgcag	aactgataga	aattggatgt	720
	gaggataaaa	ccctagcctt	caaaatgaat	ggttacatat	ccaatgcaaa	ctactcagtg	780
	aagaaagtgc	tcttcttact	cttcatcaac	catcgtctg	tagaatcaac	ttccttgaga	840
	aaagccatag	aaacagtgta	tgcagcctat	ttgccccaaa	acacacaccc	attcctgtac	900
	ctcagtttag	aaatcagttc	ccagaatgtg	gatgttaatg	tgcacccac	aaagcatgaa	960
50	gttcaacttc	tgcacgagga	gagcatcctg	gagcggtg	agcagcacat	cgagagcaag	1020
	ctcctgggct	ccaattcctc	caggatgtac	ttcaccaga	ctttgctacc	aggacttgct	1080
	ggccctctg	gggagatggt	taaatccaca	acaagtctga	cctcgtcttc	tacttctgga	1140
	agtagtgata	aggtctatgc	ccaccagatg	gttcgtacag	attcccggga	acagaagctt	1200
	gatgcatttc	tgcagcctct	gagcaaaccc	ctgtccagtc	agccccaggc	cattgtcaca	1260
55	gagataaaga	cagatatttc	tagtggcagg	gctaggcagc	aagatgagga	gatgcttgaa	1320
	ctcccagccc	ctgctgaagt	ggctgccaaa	aatcagagct	tggaggggga	tacaacaaag	1380
	gggacttcag	aaatgtcaga	gaagagagga	cctacttcca	gcaacccag	aaagagacat	1440
	cgggaagatt	ctgatgtgga	aatggtggaa	gatgattccc	gaaaggaaat	gactgcagct	1500

5 tgtaccccc ggagaaggat cattaacctc actagtgttt tgagtctcca ggaagaaatt 1560  
 aatgagcagg gacatgaggt tctccgggag atgttgcata accactcctt cgtgggctgt 1620  
 gtgaatcctc agtgggcctt ggcacagcat caaaccaagt tataccttct caacaccacc 1680  
 aagcttagtg aagaactgtt ctaccagata ctcattttatg attttgccaa ttttggtgtt 1740  
 ctcaggttat cggagccagc accgctcttt gaccttgcca tgcttgccct agatagtcca 1800  
 gagagtggct ggacagagga agatgggtccc aaagaaggac ttgctgaata cattgttgag 1860  
 tttctgaaga agaaggctga gatgcttgca gactatttct ctttggaat tgatgaggaa 1920  
 gggaacctga ttggattacc ccttctgatt gacaactatg tgccccctt ggagggactg 1980  
 cctatcttca ttcttcgact agccactgag gtgaattggg acgaagaaaa ggaatgtttt 2040  
 10 gaaagcctca gtaaagaatg cgctatgttc tattccatcc ggaagcagta catatctgag 2100  
 gagtgcagcc tctcaggcca gcagagtga gtgcctggct ccattccaaa ctctggaag 2160  
 tggactgttg aacacattgt ctataaagcc ttgcgctcac acattctgcc tctaaacat 2220  
 ttcacagaag atggaaatat cctgcagctt gctaacctgc ctgatctata caaagtcttt 2280  
 gagaggtgtt aaatatgggt atttatgcac tgtgggatgt gttcttcttt ctctgtattc 2340  
 15 cgatacaaaag tgttgatca aagtgtgata tacaaagtgt accaacataa gtgttggtag 2400  
 cacttaagac ttatacttgc cttctgatag tattccttta tacacagtgg attgattata 2460  
 aataaataga tgtgtcttaa cata 2484

## hPMS2-134 (human) (SEQ ID NO:15)

20 MERAESSSTE PAKAIKPIDR KSVHQICSGQ VVLSLSTAVK ELVENS LDAG ATNIDLKLKD 60  
 YGVDLIEVSD NGCGVEEENF EGLTLKHHTS KIQEFADLTQ VETFGFRGEA LSSL CALSDV 120  
 TISTCHASAK VGT 133

## hPMS2-134 (human cDNA) (SEQ ID NO:16)

25 cgaggcggat cgggtgttgc atccatggag cgagctgaga gctcgagtac agaacctgct 60  
 aaggccatca aacctattga tcggaagtca gtccatcaga tttgctctgg gcagggtgta 120  
 ctgagtctaa gcaactgcgg aaaggagtta gtagaaaaca gtctggatgc tgggtgccact 180  
 aatattgatc taaagcttaa ggactatgga gtggatctta ttgaagttc agacaatgga 240  
 tgtggggtag aagaagaaaa cttcgaaggc ttaactctga aacatcacac atctaagatt 300  
 30 caagagtttg ccgacctaac tcagggtgaa acttttggct ttccggggga agctctgagc 360  
 tcactttgtg cactgagcga tgtcaccatt tctacctgcc acgcatcggc gaaggttgga 420  
 actga 426

For further information on the background of the invention the following references

35 may be consulted, each of which is incorporated herein by reference in its entirety:

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The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

#### **EXAMPLE 1: Stable expression of dominant negative MMR genes in hybridoma cells**

It has been previously shown by Nicolaides et al. (Nicolaides *et al.* (1998) A Naturally Occurring hPMS2 Mutation Can Confer a Dominant Negative Mutator Phenotype *Mol. Cell. Biol.* 18:1635-1641) that the expression of a dominant negative allele in an otherwise MMR proficient cell could render these host cells MMR deficient. The creation of MMR deficient cells can lead to the generation of genetic alterations throughout the entire genome of a host organisms offspring, yielding a population of genetically altered offspring or siblings that may produce biochemicals with altered properties. This patent application teaches of the use of dominant negative MMR genes in antibody-producing cells, including but not limited to rodent hybridomas, human hybridomas, chimeric rodent cells producing human immunoglobulin gene products, human cells expressing immunoglobulin genes, mammalian cells producing single chain antibodies, and prokaryotic cells producing mammalian immunoglobulin genes or chimeric immunoglobulin molecules such as those contained within single-chain antibodies. The cell expression systems described above that are used to produce antibodies are well known by those skilled in the art of antibody therapeutics.

To demonstrate the ability to create MMR defective hybridomas using dominant negative alleles of MMR genes, we first transfected a mouse hybridoma cell line that is known to produce and antibody directed against the human IgE protein with an expression vector containing the human PMS2 (cell line referred to as HBPMS2), the previously

published dominant negative PMS2 mutant referred herein as PMS134 (cell line referred to as HB134), or with no insert (cell line referred to as HBvec). The results showed that the PMS134 mutant could indeed exert a robust dominant negative effect, resulting in biochemical and genetic manifestations of MMR deficiency. Unexpectedly was the finding that the full length PMS2 also resulted in a lower MMR activity while no effect was seen in cells containing the empty vector. A brief description of the methods is provided below.

The MMR proficient mouse H36 hybridoma cell line was transfected with various *hPMS2* expression plasmids plus reporter constructs for assessing MMR activity. The MMR genes were cloned into the pEF expression vector, which contains the elongation factor promoter upstream of the cloning site followed by a mammalian polyadenylation signal. This vector also contains the NEO<sup>r</sup> gene that allows for selection of cells retaining this plasmid. Briefly, cells were transfected with 1 µg of each vector using polyliposomes following the manufacturer's protocol (Life Technologies). Cells were then selected in 0.5 mg/ml of G418 for 10 days and G418 resistant cells were pooled together to analyze for gene expression. The pEF construct contains an intron that separates the exon 1 of the EF gene from exon 2, which is juxtaposed to the 5' end of the polylinker cloning site. This allows for a rapid reverse transcriptase polymerase chain reaction (RT-PCR) screen for cells expressing the spliced products. At day 17, 100,000 cells were isolated and their RNA extracted using the trizol method as previously described (Nicolaidis N.C., Kinzler, K.W., and Vogelstein, B. (1995) Analysis of the 5' region of PMS2 reveals heterogeneous transcripts and a novel overlapping gene. *Genomics* 29:329-334). RNAs were reverse transcribed using Superscript II (Life Technologies) and PCR amplified using a sense primer located in exon 1 of the EF gene (5'-ttt cgc aac ggg ttg gcc g-3') and an antisense primer (5'-gtt tca gag tta agc ctt cg-3') centered at nt 283 of the published human PMS2 cDNA, which will detect both the full length as well as the PMS134 gene expression. Reactions were carried out using buffers and conditions as previously described (Nicolaidis, N.C., *et al.* (1995) Genomic organization of the human PMS2 gene family. *Genomics* 30:195-206), using the following amplification parameters: 94°C for 30 sec, 52°C for 2 min, 72°C for 2 min, for 30 cycles. Reactions were analyzed on agarose gels. Figure 1 shows a representative example of PMS expression in stably transduced H36 cells.

Expression of the protein encoded by these genes were confirmed via western blot using a polyclonal antibody directed to the first 20 amino acids located in the N-terminus of the protein following the procedures previously described (data not shown) (Nicolaides *et al.* (1998) A Naturally Occurring hPMS2 Mutation Can Confer a Dominant Negative Mutator Phenotype. *Mol. Cell. Biol.* 18:1635-1641.

**EXAMPLE 2: hPMS134 Causes a Defect in MMR Activity and hypermutability in hybridoma cells**

A hallmark of MMR deficiency is the generation of unstable microsatellite repeats in the genome of host cells. This phenotype is referred to as microsatellite instability (MI) (Modrich, P. (1994) Mismatch repair, genetic stability, and cancer *Science* 266:1959-1960; Palombo, F., *et al.* (1994) Mismatch repair and cancer *Nature* 36:417). MI consists of deletions and/or insertions within repetitive mono-, di- and/or tri nucleotide repetitive sequences throughout the entire genome of a host cell. Extensive genetic analysis eukaryotic cells have found that the only biochemical defect that is capable of producing MI is defective MMR (Strand, M., *et al.* (1993) Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair *Nature* 365:274-276; Perucho, M. (1996) Cancer of the microsatellite mutator phenotype. *Biol Chem.* 377:675-684; Eshleman J.R., and Markowitz, S.D. (1996) Mismatch repair defects in human carcinogenesis. *Hum. Mol. Genet.* 5:1489-494). In light of this unique feature that defective MMR has on promoting MI, it is now used as a biochemical marker to survey for lack of MMR activity within host cells (Perucho, M. (1996) Cancer of the microsatellite mutator phenotype. *Biol Chem.* 377:675-684; Eshleman J.R., and Markowitz, S.D. (1996) Mismatch repair defects in human carcinogenesis. *Hum. Mol. Genet.* 5:1489-494; Liu, T., *et al.* (2000) Microsatellite instability as a predictor of a mutation in a DNA mismatch repair gene in familial colorectal cancer *Genes Chromosomes Cancer* 27:17-25).

A method used to detect MMR deficiency in eukaryotic cells is to employ a reporter gene that has a polynucleotide repeat inserted within the coding region that disrupts its reading frame due to a frame shift. In the case where MMR is defective, the reporter gene will acquire random mutations (i.e. insertions and/or deletions) within the polynucleotide

repeat yielding clones that contain a reporter with an open reading frame. We have employed the use of an MMR-sensitive reporter gene to measure for MMR activity in HBvec, HBPMS2, and HBPMS134 cells. The reporter construct used the pCAR-OF, which contains a hygromycin resistance (HYG) gene plus a  $\beta$ -galactosidase gene containing a 29 bp out-of-frame poly-CA tract at the 5' end of its coding region. The pCAR-OF reporter would not generate  $\beta$ -galactosidase activity unless a frame-restoring mutation (*i.e.*, insertion or deletion) arose following transfection. HBvec, HBPMS2, and HB134 cells were each transfected with pCAR-OF vector in duplicate reactions following the protocol described in Example 1. Cells were selected in 0.5 mg/ml G418 and 0.5mg/ml HYG to select for cells retaining both the MMR effector and the pCAR-OF reporter plasmids. All cultures transfected with the pCAR vector resulted in a similar number of HYG/G418 resistant cells. Cultures were then expanded and tested for  $\beta$ -galactosidase activity *in situ* as well as by biochemical analysis of cell extracts. For *in situ* analysis, 100,000 cells were harvested and fixed in 1% gluteraldehyde, washed in phosphate buffered saline solution and incubated in 1 ml of X-gal substrate solution [0.15 M NaCl, 1 mM  $MgCl_2$ , 3.3 mM  $K_4Fe(CN)_6$ , 3.3 mM  $K_3Fe(CN)_6$ , 0.2% X-Gal ] in 24 well plates for 2 hours at 37°C. Reactions were stopped in 500 mM sodium bicarbonate solution and transferred to microscope slides for analysis. Three fields of 200 cells each were counted for blue ( $\beta$ -galactosidase positive cells) or white ( $\beta$ -galactosidase negative cells) to assess for MMR inactivation. Table 1 shows the results from these studies. While no  $\beta$ -galactosidase positive cells were observed in HBvec cells, 10% of the cells per field were  $\beta$ -galactosidase positive in HB134 cultures and 2% of the cells per field were  $\beta$ -galactosidase positive in HBPMS2 cultures.

Cell extracts were prepared from the above cultures to measure  $\beta$ -galactosidase using a quantitative biochemical assay as previously described (Nicolaides *et al.* (1998)

A Naturally Occurring hPMS2 Mutation Can Confer a Dominant Negative Mutator Phenotype *Mol. Cell. Biol.* 18:1635-1641; Nicolaides, N.C., *et al.* (1992) The Jun family members, c-JUN and JUND, transactivate the human *c-myc* promoter via an Ap1 like element. *J. Biol. Chem.* 267:19665-19672). Briefly, 100,000 cells were collected, centrifuged and resuspended in 200  $\mu$ l of 0.25M Tris, pH 8.0. Cells were



lysed by freeze/thawing three times and supernatants collected after microfugation at 14,000 rpms to remove cell debris. Protein content was determined by spectrophotometric analysis at OD<sup>280</sup>. For biochemical assays, 20 µg of protein was added to buffer containing 45 mM 2-mercaptoethanol, 1mM MgCl<sub>2</sub>, 0.1 M NaPO<sub>4</sub> and 0.6 mg/ml Chlorophenol red-β-D-galactopyranoside (CPRG, Boehringer Mannheim). Reactions were incubated for 1 hour, terminated by the addition of 0.5 M Na<sub>2</sub>CO<sub>3</sub>, and analyzed by spectrophotometry at 576 nm. H36 cell lysates were used to subtract out background. Figure 2 shows the β-galactosidase activity in extracts from the various cell lines. As shown, the HB134 cells produced the highest amount of β-galactosidase, while no activity was found in the HBvec cells containing the pCAR-OF. These data demonstrate the ability to generate MMR defective hybridoma cells using dominant negative MMR gene alleles.

**Table 1.** β-galactosidase expression of HBvec, HBPMS2 and HB134 cells transfected with pCAR-OF reporter vectors. Cells were transfected with the pCAR-OF β-galactosidase reporter plasmid. Transfected cells were selected in hygromycin and G418, expanded and stained with X-gal solution to measure for β-galactosidase activity (blue colored cells). 3 fields of 200 cells each were analyzed by microscopy. The results below represent the mean +/- standard deviation of these experiments.

Table 1.

CELL LINE	# BLUE CELLS
HBvec	0 +/- 0
HBPMS2	4 +/- 1
HB134	20 +/- 3

**EXAMPLE 3: Screening strategy to identify hybridoma clones producing antibodies with higher binding affinities and/or increased immunoglobulin production.**

An application of the methods presented within this document is the use of MMR deficient hybridomas or other immunoglobulin producing cells to create genetic alterations within an immunoglobulin gene that will yield antibodies with altered biochemical properties.

An illustration of this application is demonstrated within this example whereby the HB134 hybridoma (see Example 1), which is a MMR-defective cell line that produces an anti-human immunoglobulin type E (hIgE) MAbs, is grown for 20 generations and clones are isolated in 96-well plates and screened for hIgE binding. Figure 3 outlines the screening procedure to identify clones that produce high affinity MAbs, which is presumed to be due to an alteration within the light or heavy chain variable region of the protein. The assay employs the use of a plate Enzyme Linked Immunosorbant Assay (ELISA) to screen for clones that produce high-affinity MAbs. 96-well plates containing single cells from HBvec or HB134 pools are grown for 9 days in growth medium (RPMI 1640 plus 10% fetal bovine serum) plus 0.5 mg/ml G418 to ensure clones retain the expression vector. After 9 days, plates are screened using an hIgE plate ELISA, whereby a 96 well plate is coated with 50µls of a 1µg/ml hIgE solution for 4 hours at 4°C. Plates are washed 3 times in calcium and magnesium free phosphate buffered saline solution (PBS<sup>-/-</sup>) and blocked in 100µls of PBS<sup>-/-</sup> with 5% dry milk for 1 hour at room temperature. Wells are rinsed and incubated with 100 µls of a PBS solution containing a 1:5 dilution of conditioned medium from each cell clone for 2 hours. Plates are then washed 3 times with PBS<sup>-/-</sup> and incubated for 1 hour at room temperature with 50 µls of a PBS<sup>-/-</sup> solution containing 1:3000 dilution of a sheep anti-mouse horse radish peroxidase (HRP) conjugated

secondary antibody. Plates are then washed 3 times with PBS<sup>-/-</sup> and incubated with 50 µls of TMB-HRP substrate (BioRad) for 15 minutes at room temperature to detect amount of antibody produced by each clone. Reactions are stopped by adding 50 µls of 500mM sodium bicarbonate and analyzed by OD at 415nm using a BioRad plate reader. Clones exhibiting an enhanced signal over background cells (H36 control cells) are then isolated and expanded into 10 ml cultures for additional characterization and confirmation of ELISA data in triplicate experiments. ELISAs are also performed on conditioned (CM) from the same clones to measure total Ig production within the conditioned medium of each well. Clones that produce an increased ELISA signal and have increased antibody levels are then further analyzed for variants that over-express and/or over-secrete antibodies as described in Example 4. Analysis of five 96-well plates each from HBvec or HB134 cells have found that a significant number of clones with a higher Optical Density (OD) value is observed in the MMR-defective HB134 cells as compared to the HBvec controls. Figure 4 shows a representative example of HB134 clones producing antibodies that bind to specific antigen (in this case IgE) with a higher affinity. Figure 4 provides raw data from the analysis of 96 wells of HBvec (left graph) or HB134 (right graph) which shows 2 clones from the HB134 plate to have a higher OD reading due to 1) genetic alteration of the antibody variable domain that leads to an increased binding to IgE antigen, or 2) genetic alteration of a cell host that leads to over-production/secretion of the antibody molecule. Anti-Ig ELISA found that the two clones, shown in figure 4 have Ig levels within their CM similar to the surrounding wells exhibiting lower OD values. These data suggest that a genetic alteration occurred within the antigen binding domain of the antibody which in turn allows for higher binding to antigen.

Clones that produced higher OD values as determined by ELISA were further analyzed at the genetic level to confirm that mutations within the light or heavy chain variable region have occurred that lead to a higher binding affinity hence yielding to a stronger ELISA signal. Briefly, 100,000 cells are harvested and extracted for RNA using the Triazol method as described above. RNAs are reverse transcribed using Superscript II as suggested by the manufacturer (Life Technology) and PCR amplified for the antigen binding sites contained within the variable light and heavy chains. Because of the heterogeneous nature of these

genes, the following degenerate primers are used to amplify light and heavy chain alleles from the parent H36 strain.

Light chain sense: 5'-GGA TTT TCA GGT GCA GAT TTT CAG-3' (SEQ ID NO:1)

5

Light chain antisense: 5'-ACT GGA TGG TGG GAA GAT GGA-3' (SEQ ID NO:2)

Heavy chain sense: 5'-A(G/T) GTN (A/C)AG CTN CAG (C/G)AG TC-3' (SEQ ID NO:3)

10 Heavy chain antisense: 5'-TNC CTT G(A/G)C CCC AGT A(G/A)(A/T)C-3' (SEQ ID NO:4)

PCR reactions using degenerate oligonucleotides are carried out at 94°C for 30 sec, 52°C for 1 min, and 72°C for 1 min for 35 cycles. Products are analyzed on agarose gels. Products of the expected molecular weights are purified from the gels by Gene Clean (Bio 15 101), cloned into T-tailed vectors, and sequenced to identify the wild type sequence of the variable light and heavy chains. Once the wild type sequence has been determined, non-degenerate primers were made for RT-PCR amplification of positive HB134 clones. Both the light and heavy chains were amplified, gel purified and sequenced using the corresponding sense and antisense primers. The sequencing of RT-PCR products gives representative 20 sequence data of the endogenous immunoglobulin gene and not due to PCR induced mutations. Sequences from clones were then compared to the wild type sequence for sequence comparison. An example of the ability to create *in vivo* mutations within an immunoglobulin light or heavy chain is shown in figure 5, where HB134 clone92 was identified by ELISA to have an increased signal for hIgE. The light chain was amplified 25 using specific sense and antisense primers. The light chain was RT-PCR amplified and the resulting product was purified and analyzed on an automated ABI377 sequencer. As shown in clone A, a residue -4 upstream of the CDR region 3 had a genetic change from ACT to TCT, which results in a Thr to Ser change within the framework region just preceding the CDR#3. In clone B, a residue -6 upstream of the CDR region had a genetic change from CCC to CTC, 30 which results in a Pro to His change within framework region preceding CDR#2.

The ability to generate random mutations in immunoglobulin genes or chimeric immunoglobulin genes is not limited to hybridomas. Nicolaides et al. (Nicolaides *et al.* (1998) A Naturally Occurring hPMS2 Mutation Can Confer a Dominant Negative Mutator Phenotype *Mol. Cell. Biol.* 18:1635-1641) has previously shown the ability to generate hypermutable hamster cells and produce mutations within an endogenous gene. A common method for producing humanized antibodies is to graft CDR sequences from a MAb (produced by immunizing a rodent host) onto a human Ig backbone, and transfection of the chimeric genes into Chinese Hamster Ovary (CHO) cells which in turn produce a functional Ab that is secreted by the CHO cells (Shields, R.L., *et al.* (1995) Anti-IgE monoclonal antibodies that inhibit allergen-specific histamine release. *Int. Arch. Allergy Immunol.* 107:412-413). The methods described within this application are also useful for generating genetic alterations within Ig genes or chimeric Igs transfected within host cells such as rodent cell lines, plants, yeast and prokaryotes (Frigerio L, *et al.* (2000) Assembly, secretion, and vacuolar delivery of a hybrid immunoglobulin in plants. *Plant Physiol.* 123:1483-1494).

These data demonstrate the ability to generate hypermutable hybridomas, or other Ig producing host cells that can be grown and selected, to identify structurally altered immunoglobulins yielding antibodies with enhanced biochemical properties, including but not limited to increased antigen binding affinity. Moreover, hypermutable clones that contain missense mutations within the immunoglobulin gene that result in an amino acid change or changes can be then further characterized for *in vivo* stability, antigen clearance, on-off binding to antigens, etc. Clones can also be further expanded for subsequent rounds of *in vivo* mutations and can be screened using the strategy listed above.

The use of chemical mutagens to produce genetic mutations in cells or whole organisms are limited due to the toxic effects that these agents have on "normal" cells. The use of chemical mutagens such as MNU in MMR defective organisms is much more tolerable yielding to a 10 to 100 fold increase in genetic mutation over MMR deficiency alone (Bignami M, (2000) Unmasking a killer: DNA O(6)-methylguanine and the cytotoxicity of methylating agents. *Mutat. Res.* 462:71-82). This strategy allows for the use of chemical mutagens to be used in MMR-defective Ab producing cells as a method for increasing

additional mutations within immunoglobulin genes or chimeras that may yield functional Abs with altered biochemical properties such as enhanced binding affinity to antigen, etc.

**Example 4: Generation of antibody producing cells with enhanced antibody production**

Analysis of clones from H36 and HB134 following the screening strategy listed above has identified a significant number of clones that produce enhanced amounts of antibody into the medium. While a subset of these clones gave higher Ig binding data as determined by ELISA as a consequence of mutations within the antigen binding domains contained in the variable regions, others were found to contain "enhanced" antibody production. A summary of the clones producing enhanced amounts of secreted MAb is shown in TABLE 2, where a significant number of clones from HB134 cells were found to produce enhanced Ab production within the conditioned medium as compared to H36 control cells.

**TABLE 2.** Generation of hybridoma cells producing high levels of antibody. HB134 clones were assayed by ELISA for elevated Ig levels. Analysis of 480 clones showed that a significant number of clones had elevated MAb product levels in their CM. Quantification showed that several of these clones produced greater than 500ng/ml of MAb due to either enhanced expression and/or secretion as compared to clones from the H36 cell line.

**Table 2. Production of MAb in CM from H36 and HB134 clones.**

Cell Line	% clones > 400 ng/ml	% clones >500 ng/ml
H36	1/480 = 0.2%	0/480 = 0%
HB134	50/480 = 10%	8/480 = 1.7%

Cellular analysis of HB134 clones with higher MAb levels within the conditioned medium (CM) were analyzed to determine if the increased production was simply due to genetic alterations at the Ig locus that may lead to over-expression of the polypeptides forming the antibody, or due to enhanced secretion due to a genetic alteration affecting secretory pathway mechanisms. To address this issue, we expanded three HB134 clones that had increased levels of antibody within their CM. 10,000 cells were prepared for western blot

analysis to assay for intracellular steady state Ig protein levels (Figure 6). In addition, H36 cells were used as a standard reference (Lane 2) and a rodent fibroblast (Lane 1) was used as an Ig negative control. Briefly, cells were pelleted by centrifugation and lysed directly in 300 µl of SDS lysis buffer (60 mM Tris, pH 6.8, 2% SDS, 10% glycerol, 0.1 M

5 2-mercaptoethanol, 0.001% bromophenol blue) and boiled for 5 minutes. Lysate proteins were separated by electrophoresis on 4-12% NuPAGE gels (for analysis of Ig heavy chain. Gels were electroblotted onto Immobilon-P (Millipore) in 48 mM Tris base, 40 mM glycine, 0.0375% SDS, 20% methanol and blocked at room temperature for 1 hour in Tris-buffered saline (TBS) plus 0.05% Tween-20 and 5% condensed milk. Filters were probed with a  
10 1:10,000 dilution of sheep anti-mouse horseradish peroxidase conjugated monoclonal antibody in TBS buffer and detected by chemiluminescence using Supersignal substrate (Pierce). Experiments were repeated in duplicates to ensure reproducibility. Figure 6 shows a representative analysis where a subset of clones had enhanced Ig production which accounted for increased Ab production (Lane 5) while others had a similar steady state level as the  
15 control sample, yet had higher levels of Ab within the CM. These data suggest a mechanism whereby a subset of HB134 clones contained a genetic alteration that in turn produces elevated secretion of antibody.

The use of chemical mutagens to produce genetic mutations in cells or whole organisms are limited due to the toxic effects that these agents have on “normal” cells. The  
20 use of chemical mutagens such as MNU in MMR defective organisms is much more tolerable yielding to a 10 to 100 fold increase in genetic mutation over MMR deficiency alone (Bignami M, (2000) Unmasking a killer: DNA O(6)-methylguanine and the cytotoxicity of methylating agents. *Mutat. Res.* 462:71-82). This strategy allows for the use of chemical mutagens to be used in MMR-defective Ab producing cells as a method for increasing  
25 additional mutations within immunoglobulin genes or chimeras that may yield functional Abs with altered biochemical properties such as enhanced binding affinity to antigen, etc.

#### **Example 5: establishment of genetic stability in hybridoma cells with new output trait.**

The initial steps of MMR are dependent on two protein complexes, called MutSα and  
30 MutLα (Nicolaidis *et al.* (1998) A Naturally Occurring hPMS2 Mutation Can Confer a

Dominant Negative Mutator Phenotype. *Mol. Cell. Biol.* 18:1635-1641). Dominant negative MMR alleles are able to perturb the formation of these complexes with downstream biochemicals involved in the excision and polymerization of nucleotides comprising the “corrected” nucleotides. Examples from this application show the ability of a truncated MMR allele (PMS134) as well as a full length human PMS2 when expressed in a hybridoma cell line is capable of blocking MMR resulting in a hypermutable cell line that gains genetic alterations throughout its entire genome per cell division. Once a cell line is produced that contains genetic alterations within genes encoding for an antibody, a single chain antibody, over expression of immunoglobulin genes and/or enhanced secretion of antibody, it is desirable to restore the genomic integrity of the cell host. This can be achieved by the use of inducible vectors whereby dominant negative MMR genes are cloned into such vectors, introduced into Ab producing cells and the cells are cultured in the presence of inducer molecules and/or conditions. Inducible vectors include but are not limited to chemical regulated promoters such as the steroid inducible MMTV, tetracycline regulated promoters, temperature sensitive MMR gene alleles, and temperature sensitive promoters.

The results described above lead to several conclusions. First, expression of hPMS2 and PMS134 results in an increase in microsatellite instability in hybridoma cells. That this elevated microsatellite instability is due to MMR deficiency was proven by evaluation of extracts from stably transduced cells. The expression of PMS134 results in a polar defect in MMR, which was only observed using heteroduplexes designed to test repair from the 5' direction (no significant defect in repair from the 3' direction was observed in the same extracts) (Nicolaidis *et al.* (1998) A Naturally Occurring hPMS2 Mutation Can Confer a Dominant Negative Mutator Phenotype. *Mol. Cell. Biol.* 18:1635-1641). Interestingly, cells deficient in hMLH1 also have a polar defect in MMR, but in this case preferentially affecting repair from the 3' direction (Drummond, J.T, *et al.* (1996) Cisplatin and adriamycin resistance are associated with MutLa and mismatch repair deficiency in an ovarian tumor cell line. *J. Biol. Chem.* 271:9645-19648). It is known from previous studies in both prokaryotes and eukaryotes that the separate enzymatic components mediate repair from the two different directions. Our results, in combination with those of Drummond *et al.* (Shields, R.L., *et al.* (1995) Anti-IgE monoclonal antibodies that inhibit allergen-specific histamine release. *Int.*



*Arch Allergy Immunol.* 107:412-413), strongly suggest a model in which 5' repair is primarily dependent on hPMS2 while 3' repair is primarily dependent on hMLH1. It is easy to envision how the dimeric complex between PMS2 and MLH1 might set up this directionality. The combined results also demonstrate that a defect in directional MMR is sufficient to produce a MMR defective phenotype and suggests that any MMR gene allele is useful to produce genetically altered hybridoma cells, or a cell line that is producing Ig gene products. Moreover, the use of such MMR alleles will be useful for generating genetically altered Ig polypeptides with altered biochemical properties as well as cell hosts that produce enhanced amounts of antibody molecules.

Another method that is taught in this application is that ANY method used to block MMR can be performed to generate hypermutability in an antibody-producing cell that can lead to genetically altered antibodies with enhanced biochemical features such as but not limited to increased antigen binding, enhanced pharmacokinetic profiles, etc. These processes can also be used to generate antibody producer cells that have increased Ig expression as shown in Example 4, figure 6 and/or increased antibody secretion as shown in Table 2.

In addition, we demonstrate the utility of blocking MMR in antibody producing cells to increase genetic alterations within Ig genes that may lead to altered biochemical features such as, but not limited to, increased antigen binding affinities (Figure 5A and 5B). The blockade of MMR in such cells can be through the use of dominant negative MMR gene alleles from any species including bacteria, yeast, protozoa, insects, rodents, primates, mammalian cells, and man. Blockade of MMR can also be generated through the use of antisense RNA or deoxynucleotides directed to any of the genes involved in the MMR biochemical pathway. Blockade of MMR can be through the use of polypeptides that interfere with subunits of the MMR complex including but not limited to antibodies. Finally, the blockade of MMR may be through the use chemicals such as but not limited to nonhydrolyzable ATP analogs, which have been shown to block MMR (Galio, L, *et al.* (1999) ATP hydrolysis-dependent formation of a dynamic ternary nucleoprotein complex with MutS and MutL. *Nucl. Acids Res.* 27:2325-23231).

## WE CLAIM:

1. A method for making a hypermutable, antibody producing cell, comprising introducing into a cell capable of producing antibodies a polynucleotide comprising a dominant negative allele of a mismatch repair gene.
2. The method of claim 1 wherein said polynucleotide is introduced by transfection of a suspension of cells *in vitro*.
3. The method of claim 1 wherein said mismatch repair gene is *PMS2*.
4. The method of claim 1 wherein said mismatch repair gene is human *PMS2*.
5. The method of claim 1 wherein said mismatch repair gene is *MLH1*.
6. The method of claim 1 wherein said mismatch repair gene is *PMS1*.
7. The method of claim 1 wherein said mismatch repair gene is *MSH2*.
8. The method of claim 1 wherein said mismatch repair gene is *MSH2*.
9. The method of claim 4 wherein said allele comprises a truncation mutation.
10. The method of claim 4 wherein said allele comprises a truncation mutation at codon 134.
11. The method of claim 10 wherein said truncation mutation is a thymidine at nucleotide 424 of wild-type *PMS2*.
12. The method of claim 1 wherein said polynucleotide is introduced into a fertilized egg of an animal.
13. The method of claim 12 wherein the fertilized egg is subsequently implanted into a pseudo-pregnant female whereby the fertilized egg develops into a mature transgenic animal.
14. The method of claim 12 wherein said mismatch repair gene is *PMS2*.
15. The method of claim 12 wherein said mismatch repair gene is human *PMS2*.
16. The method of claim 12 wherein said mismatch repair gene is human *MLH1*.
17. The method of claim 12 wherein said mismatch repair gene is human *PMS1*.
18. The method of claim 11 wherein said mismatch repair gene is a human *mutL* homolog.
19. The method of claim 15 wherein said allele comprises a truncation mutation.
20. The method of claim 15 wherein said allele comprises a truncation mutation at codon 134.

21. The method of claim 19 wherein said truncation mutation is a thymidine at nucleotide 424 of wild-type *PMS2*.
22. The method of claim 1 wherein said capability is due to the co-introduction of an immunoglobulin gene into said cell.
23. A homogeneous culture of hypermutable, mammalian cells wherein said cells comprise a dominant negative allele of a mismatch repair gene.
24. The culture of hypermutable, mammalian cells of claim 23 wherein the mismatch repair gene is *PMS2*.
25. The culture of hypermutable, mammalian cells of claim 24 wherein the mismatch repair gene is human *PMS2*.
26. The culture of hypermutable, mammalian cells of claim 23 wherein the mismatch repair gene is *MLH1*.
27. The culture of hypermutable, mammalian cells of claim 23 wherein the mismatch repair gene is *PMS1*.
28. The culture of hypermutable, mammalian cells of claim 23 wherein the mismatch repair gene is a human *mutL* homolog.
29. The culture of hypermutable, mammalian cells of claim 23 wherein the cells express a protein consisting of the first 133 amino acids of hPMS2.
30. A method for generating a mutation in a gene affecting antibody production in an antibody-producing cell comprising:
  - growing a said cell comprising said gene and a dominant negative allele of a mismatch repair gene; and
  - testing the cell to determine whether said gene of interest harbors a mutation.
31. The method of claim 30 wherein the step of testing comprises analyzing a nucleotide sequence of said gene.
32. The method of claim 30 wherein the step of testing comprises analyzing mRNA transcribed from said gene.
33. The method of claim 30 wherein the step of testing comprises analyzing a protein encoded by the gene of interest.

34. The method of claim 30 wherein the step of testing comprises analyzing the phenotype of said gene.
35. The method of claim 30 wherein the step of testing comprises analyzing the binding activity of an antibody.
36. A method wherein a mammalian cell is made MMR defective by the process of introducing a polynucleotide comprising an antisense oligonucleotide targeted against an allele of a mismatch repair gene into a mammalian cell, whereby the cell becomes hypermutable.
37. The method of claim 36 wherein the step of testing comprises analyzing a nucleotide sequence of said gene.
38. The method of claim 36 wherein the step of testing comprises analyzing mRNA transcribed from said gene.
39. The method of claim 36 wherein the step of testing comprises analyzing a protein encoded by said gene.
40. The method of claim 36 wherein the step of testing comprises analyzing the phenotype of said gene.
41. The method of claim 36 wherein the step of testing comprises analyzing the binding activity of an antibody.
42. A method for generating a mutation in a gene affecting antibody production in an antibody-producing cell comprising:
  - growing said cell comprising said gene and a polynucleotide encoding a dominant negative allele of a mismatch repair gene; and
  - testing said cell to determine whether said cell harbors at least one mutation in said gene yielding to a new biochemical feature to the product of said gene, wherein said new biochemical feature is selected from the group consisting of over-expression of said product, enhanced secretion of said product, enhanced affinity of said product for antigen, and combinations thereof.
43. The method of claim 42 wherein the step of testing comprises analyzing the steady state expression of the immunoglobulin gene of said cell.

44. The method of claim 42 wherein the step of testing comprises analyzing steady state mRNA transcribed from the immunoglobulin gene of said cell.
45. The method of claim 42 wherein the step of testing comprises analyzing the amount of secreted protein encoded by the immunoglobulin gene of said cell.
46. The method of claim 36 wherein the cell is made by the process of introducing a polynucleotide comprising a dominant negative allele of a mismatch repair gene into a cell in the presence of DNA mutagens.
47. The method of claim 46 wherein the step of testing comprises analyzing a nucleotide sequence of an immunoglobulin gene of said cell.
48. The method of claim 46 wherein the step of testing comprises analyzing mRNA transcribed from the immunoglobulin gene of said cell.
49. The method of claim 46 wherein the step of testing comprises analyzing the immunoglobulin protein encoded by said gene.
50. The method of claim 46 wherein the step of testing comprises analyzing the biochemical activity of the protein encoded by said gene.
51. A hypermutable transgenic mammalian cell made by the method of claim 42.
52. The transgenic mammalian cell of claim 51 wherein said cell is from primate.
53. The transgenic mammalian cell of claim 51 wherein said cell is from rodent.
54. The transgenic mammalian cell of claim 51 wherein said cell is from human.
55. The transgenic mammalian cell of claim 51 wherein said cell is eukaryotic.
56. The transgenic mammalian cell of claim 51 wherein said cell is prokaryotic.
57. A method of reversibly altering the hypermutability of an antibody producing cell comprising introducing an inducible vector into a cell, wherein said inducible vector comprises a dominant negative allele of a mismatch repair gene operably linked to an inducible promoter, and inducing said cell to express said dominant negative mismatch repair gene.
58. The method of claim 57 wherein said mismatch repair gene is *PMS2*.
59. The method of claim 58 wherein said mismatch repair gene is human *PMS2*.
60. The method of claim 57 wherein said mismatch repair gene is *MLH1*.
61. The method of claim 57 wherein said mismatch repair gene is *PMS1*.

62. The method of claim 57 wherein said mismatch repair gene is a human *mutL* homolog.
63. The method of claim 57 wherein said cell expresses a protein consisting of the first 133 amino acids of hPMS2.
64. The method of claim 57 further comprising analyzing the immunoglobulin protein expressed by said antibody producing cell.
65. The method of claim 64 further comprising ceasing induction of said cell, thereby restoring genetic stability of said cell.
66. A method of producing genetically altered antibodies comprising
  - transfecting a polynucleotide encoding an immunoglobulin protein into a cell, wherein said cell comprises a dominant negative mismatch repair gene;
  - growing said cell, thereby producing a hypermutated polynucleotide encoding a hypermutated immunoglobulin protein;
  - screening for a desirable property of said hypermutated immunoglobulin protein;
  - isolating said hypermutated polynucleotide; and
  - transfecting said hypermutated polynucleotide into a genetically stable cell, thereby producing a hypermutated antibody-producing, genetically stable cell.
67. The method of claim 66 wherein said mismatch repair gene is *PMS2*.
68. The method of claim 66 wherein said mismatch repair gene is human *PMS2*.
69. The method of claim 66 wherein said mismatch repair gene is *MLH1*.
70. The method of claim 66 wherein said mismatch repair gene is *PMS1*.
71. The method of claim 66 wherein said mismatch repair gene is a human *mutL* homolog.
72. The method of claim 66 wherein said cell expresses a protein consisting of the first 133 amino acids of hPMS2.

**ABSTRACT**

Dominant negative alleles of human mismatch repair genes can be used to generate hypermutable cells and organisms. By introducing these genes into cells and transgenic animals, new cell lines and animal varieties with novel and useful properties can be prepared more efficiently than by relying on the natural rate of mutation. These methods are useful for generating genetic diversity within immunoglobulin genes directed against an antigen of interest to produce altered antibodies with enhanced biochemical activity. Moreover, these methods are useful for generating antibody-producing cells with increased level of antibody production.

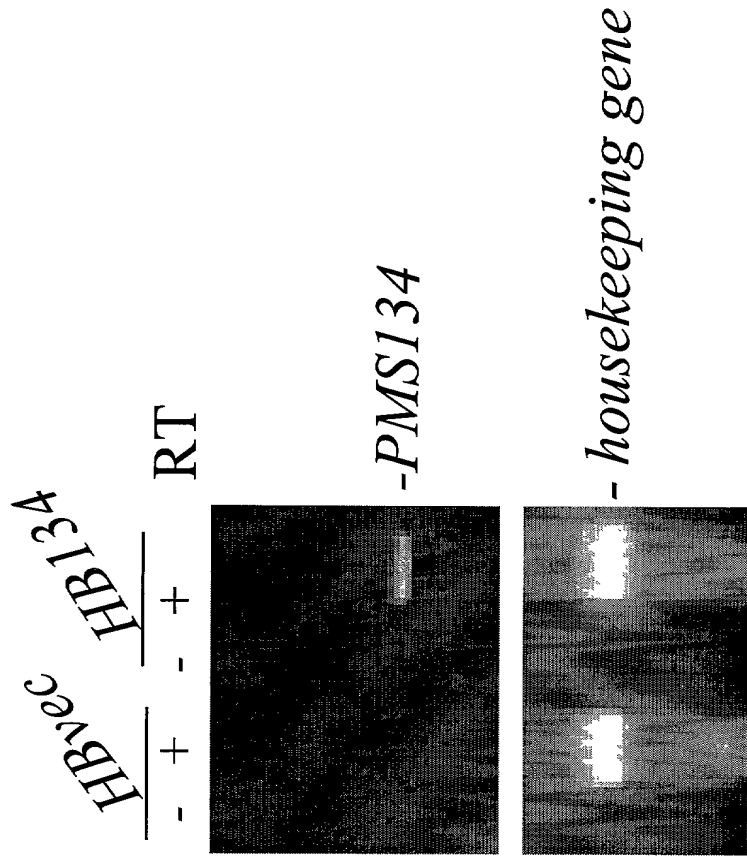


FIG. 1/6



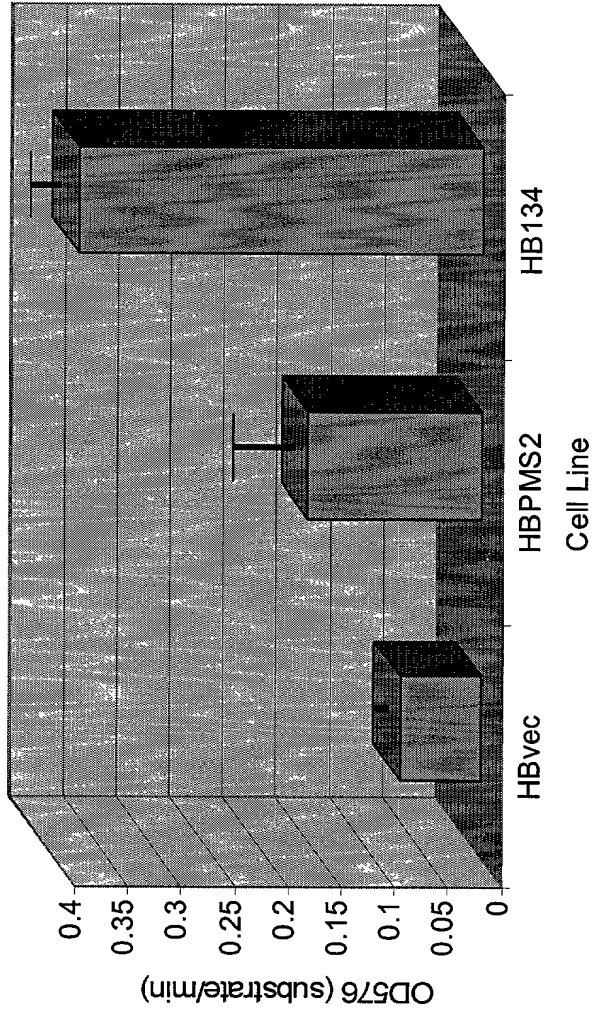


Fig. 2/6

Fig. 3/6

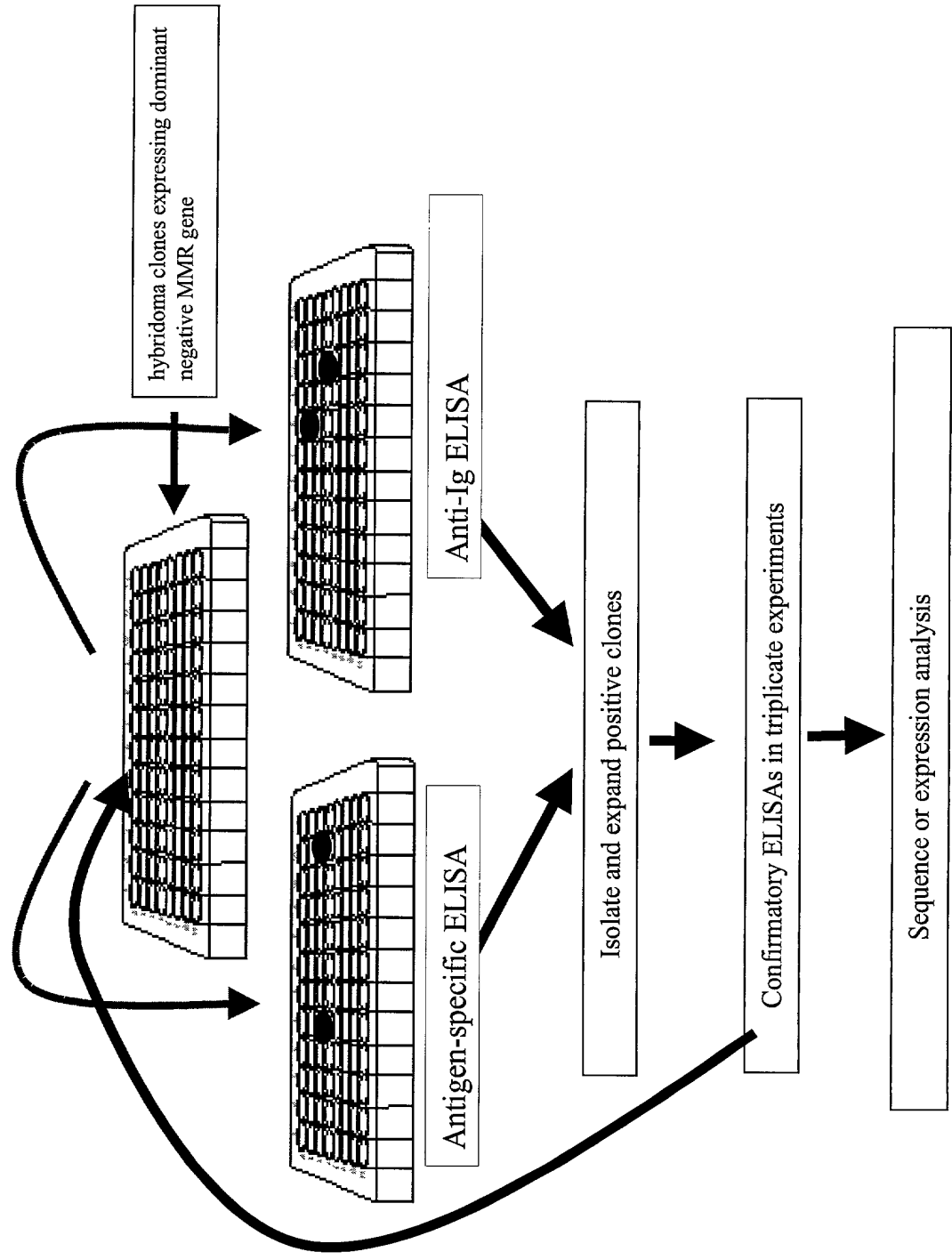
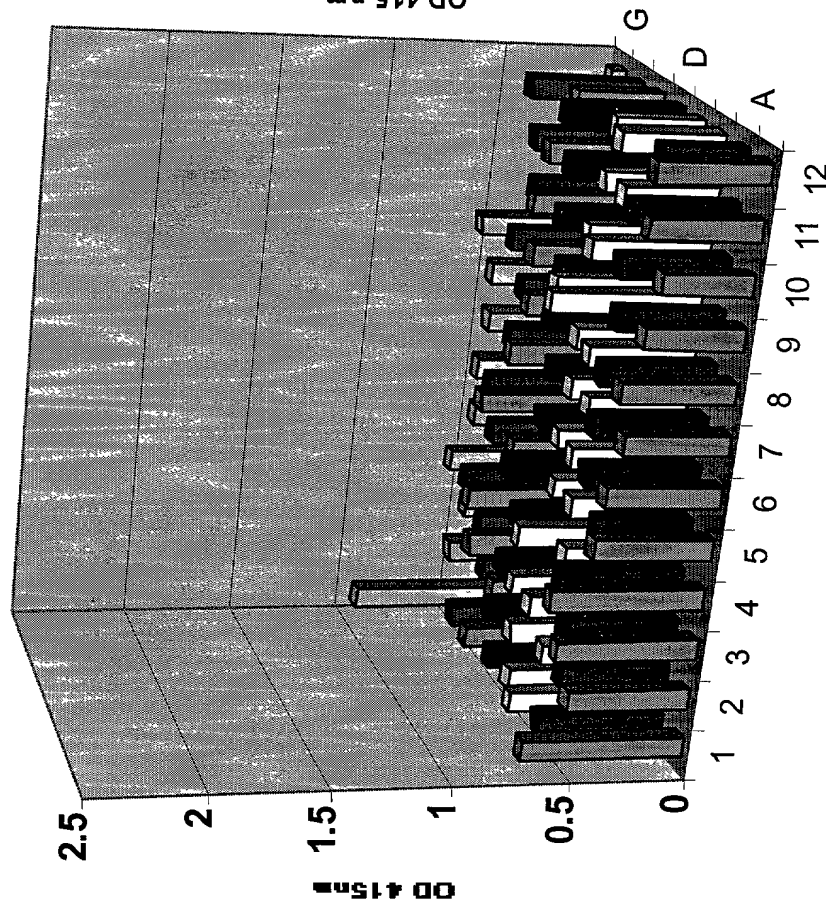
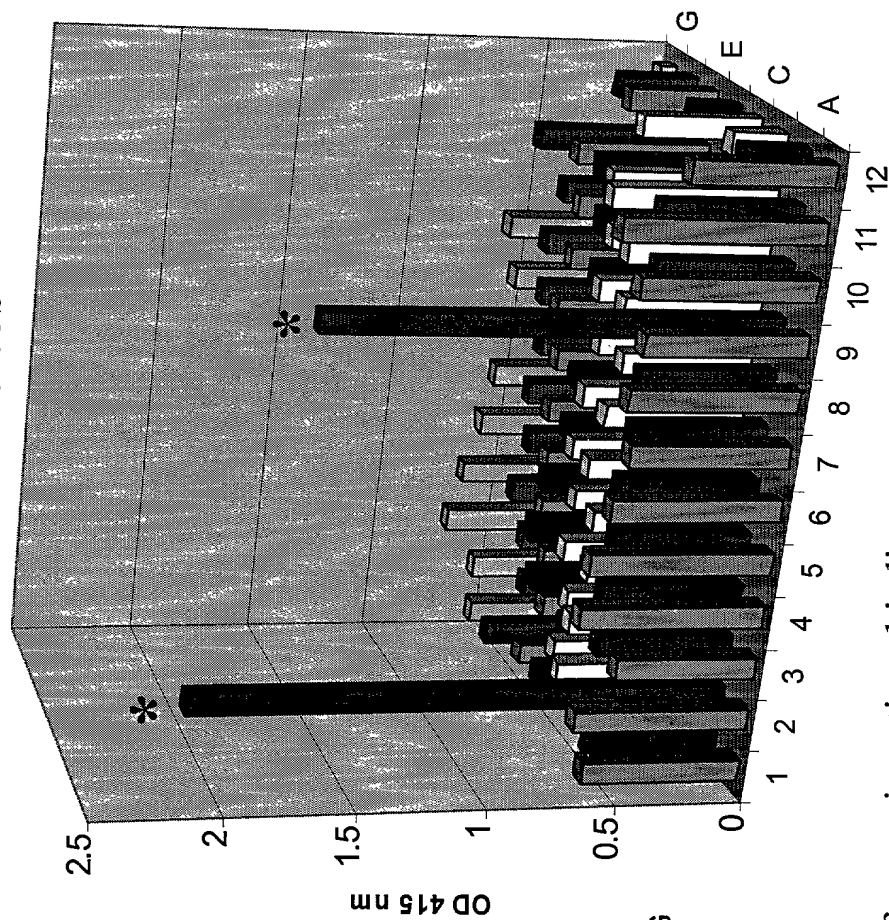


Fig.4/6

*HBvec clones*

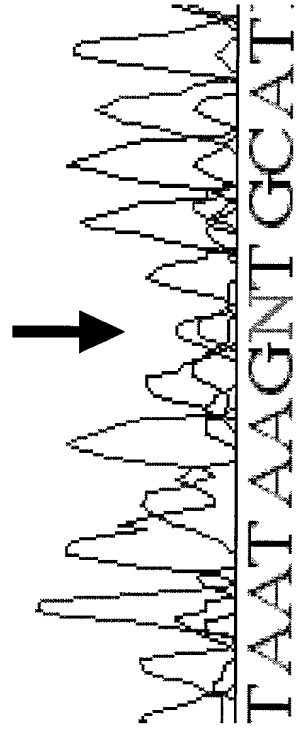


*HB134 clones*



\* = clones with a significant difference in antigen binding

HB134



Consensus sequence

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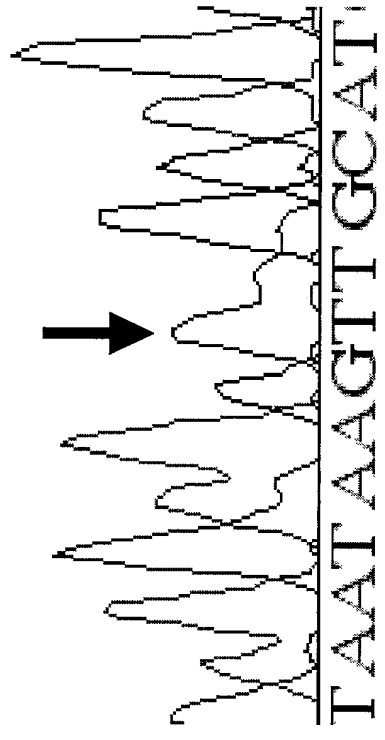
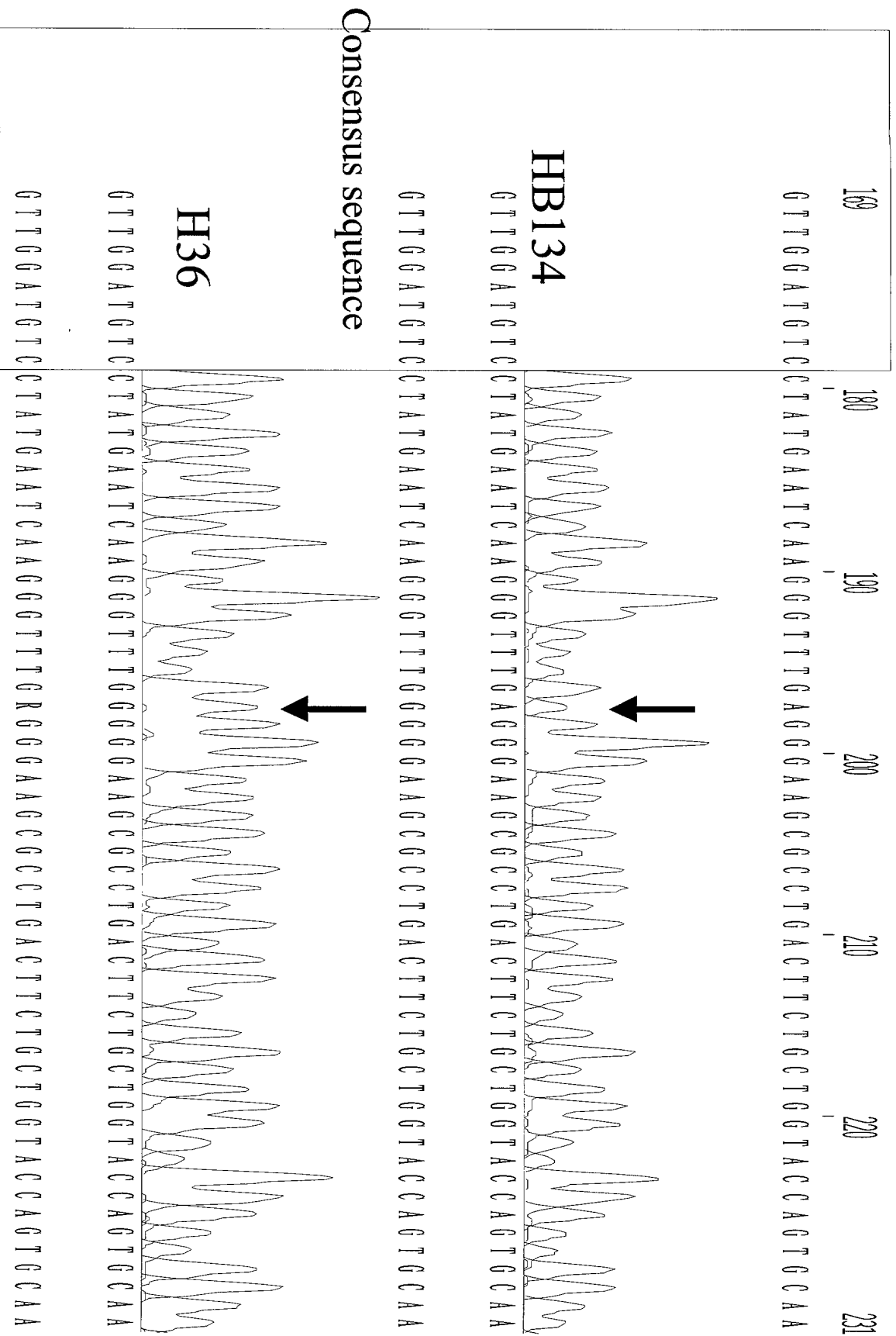
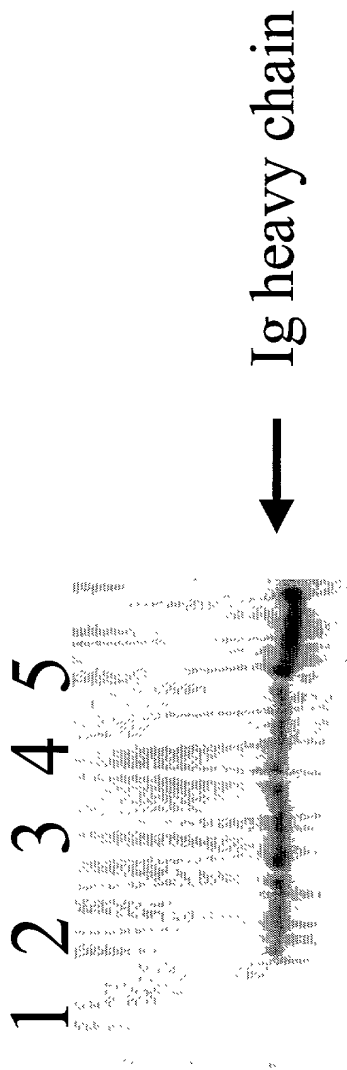


Fig. 5A/6



**Fig. 5B/6**



**Fig. 6/6**

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

**In Re Application of:**

Nicholas C. Nicolaides, Luigi Grasso, and Philip  
M. Sass

**Group Art Unit:** Not assigned**Examiner:** Not assigned

**For:** METHODS FOR GENERATING  
GENETICALLY ALTERED ANTIBODY-  
PRODUCING CELL LINES WITH  
IMPROVED ANTIBODY  
CHARACTERISTICS

**DECLARATION AND POWER OF ATTORNEY**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a

☒ Utility Patent      ☐ Design Patent

is sought on the invention, whose title appears above, the specification of which:

☒ is attached hereto.  
☐ was filed on \_\_\_\_\_ as Serial No. \_\_\_\_\_ .  
☐ said application having been amended on \_\_\_\_\_ .

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to be material to the patentability of this application in accordance with 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a-d) of any **foreign application(s)** for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of any application on which priority is claimed:

Priority Claimed (If X'd)	Country	Serial Number	Date Filed
<input type="checkbox"/>	_____	_____	_____
<input type="checkbox"/>	_____	_____	_____
<input type="checkbox"/>	_____	_____	_____
<input type="checkbox"/>	_____	_____	_____

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to be material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Serial Number	Date Filed	Patented/Pending/Abandoned
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

Serial Number	Date Filed
_____	_____



I hereby appoint the following persons of the firm of **WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS LLP**, One Liberty Place - 46th Floor, Philadelphia, Pennsylvania 19103 as attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

John W. Caldwell Reg. No. 28,937

Patrick J. Farley Reg. No. 42,524

Address all telephone calls and correspondence to:

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**WOODCOCK WASHBURN KURTZ  
MACKIEWICZ & NORRIS LLP**  
One Liberty Place - 46th Floor  
Philadelphia PA 19103  
Telephone No.: **(215) 568-3100**  
Facsimile No.: **(215) 568-3439**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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<b>Mailing Address:</b> 4 Cider Mill Court Boothwyn, PA 19061	<b>Signature</b>  <b>Date of Signature:</b> _____
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<b>Name:</b> Luigi Grasso	
<b>Mailing Address:</b> 834 Chestnut Street, Apt#816 Philadelphia, PA 19107	<b>Signature</b>  <b>Date of Signature:</b> _____
<b>City/State of Actual Residence:</b> Philadelphia, Pennsylvania	<b>Citizenship:</b> <u>United States</u>

<b>Name:</b> Philip M. Sass	
<b>Mailing Address:</b> 1903 Blackhawk Circle Audubon, PA 19403	<b>Signature</b>  <b>Date of Signature:</b> _____
<b>City/State of Actual Residence:</b> Audubon, Pennsylvania	<b>Citizenship:</b> <u>United States</u>

<b>Name:</b>	
<b>Mailing Address:</b>	<b>Signature</b>  <b>Date of Signature:</b> _____
<b>City/State of Actual Residence:</b>	<b>Citizenship:</b> _____

2025-03-14 10:00:00

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

**In Re Application of:**

Nicholas C. Nicolaides, Luigi Grasso, and  
Philip M. Sass

**Serial No.:** Not assigned**Group Art Unit:** Not assigned**Filing Date:** November 7, 2000**Examiner:** Not assigned

**For:** METHODS FOR GENERATING GENETICALLY ALTERED ANTIBODY-  
PRODUCING CELL LINES WITH IMPROVED ANTIBODY  
CHARACTERISTICS

**BOX SEQUENCE**


Assistant Commissioner for Patents  
Washington DC 20231

**STATEMENT TO SUPPORT FILING AND SUBMISSION IN ACCORDANCE  
WITH 37 CFR §§ 1.821 THROUGH 1.825**

- ☒ I hereby state, in accordance with the requirements of 37 C.F.R. §1.821(f), that the contents of the paper and computer readable copies of the Sequence Listing, submitted in accordance with 37 CFR §1.821(c) and (e), respectively are the same.
- ☐ I hereby state that the submission filed in accordance with 37 CFR §1.821(g) does not include new matter.
- ☐ I hereby state that the submission filed in accordance with 37 CFR §1.821(h) does not include new matter or go beyond the disclosure in the international application as filed.
- ☐ I hereby state that the amendments, made in accordance with 37 CFR §1.825(a), included in the substitute sheet(s) of the Sequence Listing are supported in the application, as filed, at pages \_\_\_\_\_. I hereby state that the substitute sheet(s) of the Sequence Listing does (do) not include new matter.
- ☐ I hereby state that the substitute copy of the computer readable form, submitted in accordance with 37 CFR §1.825(b), is the same as the amended Sequence Listing.

☐ I hereby state that the substitute copy of the computer readable form, submitted in accordance with 37 CFR §1.825(d), contains identical data to that originally filed.

Date: 11/7/00

  
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[illegible]

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ANTIBODY-PRODUCING CELL LINES WITH IMPROVED ANTIBODY  
CHARACTERISTICS

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&lt;170&gt; PatentIn Ver. 2.1

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Glu Lys Tyr Asn Phe Glu Met Leu Gln Gln His Thr Val Leu Gln Gly 705	710	715 720
Gln Arg Leu Ile Ala Pro Gln Thr Leu Asn Leu Thr Ala Val Asn Glu 725	730	735
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Phe Val Ile Asp Glu Asn Ala Pro Val Thr Glu Arg Ala Lys Leu Ile 755	760	765
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Ser Arg Val Lys Gln Met Phe Ala Ser Arg Ala Cys Arg Lys Ser Val 805	810	815
Met Ile Gly Thr Ala Leu Asn Thr Ser Glu Met Lys Lys Leu Ile Thr 820	825	830
His Met Gly Glu Met Asp His Pro Trp Asn Cys Pro His Gly Arg Pro 835	840	845
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<211> 2771

<212> DNA

<213> Homo sapiens

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Ser	Asn	Asn	Asn	Tyr	Pro	Ile	Pro	Glu	Gln	Met	Asn	Leu	Asn	Glu	Asp	530	535	540
Ser	Cys	Asn	Lys	Lys	Ser	Asn	Val	Ile	Asp	Asn	Lys	Ser	Gly	Lys	Val	545	550	555
Thr	Ala	Tyr	Asp	Leu	Leu	Ser	Asn	Arg	Val	Ile	Lys	Lys	Pro	Met	Ser	565	570	575
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Trp	Lys	Thr	Leu	Ser	Glu	Glu	Glu	Lys	Leu	Lys	Tyr	Glu	Glu	Lys	Ala	610	615	620
Thr	Lys	Asp	Leu	Glu	Arg	Tyr	Asn	Ser	Gln	Met	Lys	Arg	Ala	Ile	Glu	625	630	635
Gln	Glu	Ser	Gln	Met	Ser	Leu	Lys	Asp	Gly	Arg	Lys	Lys	Ile	Lys	Pro	645	650	655
Thr	Ser	Ala	Trp	Asn	Leu	Ala	Gln	Lys	His	Lys	Leu	Lys	Thr	Ser	Leu	660	665	670
Ser	Asn	Gln	Pro	Lys	Leu	Asp	Glu	Leu	Leu	Gln	Ser	Gln	Ile	Glu	Lys	675	680	685
Arg	Arg	Ser	Gln	Asn	Ile	Lys	Met	Val	Gln	Ile	Pro	Phe	Ser	Met	Lys	690	695	700
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Lys	Asp	Glu	Pro	Cys	Leu	Ile	His	Asn	Leu	Arg	Phe	Pro	Asp	Ala	Trp	725	730	735
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785 790 795 800

Arg Tyr Ser Gly Ser Thr Tyr Leu Ser Asp Pro Arg Leu Thr Ala Asn  
805 810 815

Gly Phe Lys Ile Lys Leu Ile Pro Gly Val Ser Ile Thr Glu Asn Tyr  
820 825 830

Leu Glu Ile Glu Gly Met Ala Asn Cys Leu Pro Phe Tyr Gly Val Ala  
835 840 845

Asp Leu Lys Glu Ile Leu Asn Ala Ile Leu Asn Arg Asn Ala Lys Glu  
850 855 860

Val Tyr Glu Cys Arg Pro Arg Lys Val Ile Ser Tyr Leu Glu Gly Glu  
865 870 875 880

Ala Val Arg Leu Ser Arg Gln Leu Pro Met Tyr Leu Ser Lys Glu Asp  
885 890 895

Ile Gln Asp Ile Ile Tyr Arg Met Lys His Gln Phe Gly Asn Glu Ile  
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Glu	Phe	Pro	Asp	Asn	Asp	Gln	Phe	Ser	Asn	Leu	Glu	Ala	Leu	Leu	Ile	
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Val Leu Pro Glu Met Glu Asn Gln Val Ala Val Ser Ser Leu Ser Ala						
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Val Ile Lys Phe Leu Glu Leu Leu Ser Asp Asp Ser Asn Phe Gly Gln						
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		895
Thr Glu Met Ser Glu Glu Asn Ile Thr Ile Lys Leu Lys Gln Leu Lys		
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Val Ile Val Lys Glu Gly Gly Leu Lys Leu Ile Gln Ile Gln Asp Asn  
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Gly Thr Gly Ile Arg Lys Glu Asp Leu Asp Ile Val Cys Glu Arg Phe  
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Thr Thr Ser Lys Leu Gln Ser Phe Glu Asp Leu Ala Ser Ile Ser Thr  
85 90 95

Tyr Gly Phe Arg Gly Glu Ala Leu Ala Ser Ile Ser His Val Ala His  
100 105 110

Val Thr Ile Thr Thr Lys Thr Ala Asp Gly Lys Cys Ala Tyr Arg Ala  
115 120 125

Ser Tyr Ser Asp Gly Lys Leu Lys Ala Pro Pro Lys Pro Cys Ala Gly  
130 135 140

Asn Gln Gly Thr Gln Ile Thr Val Glu Asp Leu Phe Tyr Asn Ile Ala  
145 150 155 160

Thr Arg Arg Lys Ala Leu Lys Asn Pro Ser Glu Glu Tyr Gly Lys Ile  
165 170 175

Leu Glu Val Val Gly Arg Tyr Ser Val His Asn Ala Gly Ile Ser Phe  
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Ser Val Lys Lys Gln Gly Glu Thr Val Ala Asp Val Arg Thr Leu Pro  
195 200 205

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Lys	Met	Asn	Gly	Tyr	Ile	Ser	Asn	Ala	Asn	Tyr	Ser	Val	Lys	Lys	Cys
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Ile	Phe	Leu	Leu	Phe	Ile	Asn	His	Arg	Leu	Val	Glu	Ser	Thr	Ser	Leu
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Arg	Lys	Ala	Ile	Glu	Thr	Val	Tyr	Ala	Ala	Tyr	Leu	Pro	Lys	Asn	Thr
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His	Pro	Phe	Leu	Tyr	Leu	Ser	Leu	Glu	Ile	Ser	Pro	Gln	Asn	Val	Asp
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Val	Asn	Val	His	Pro	Thr	Lys	His	Glu	Val	His	Phe	Leu	His	Glu	Glu
305					310					315					320
Ser	Ile	Leu	Glu	Arg	Val	Gln	Gln	His	Ile	Glu	Ser	Lys	Leu	Leu	Gly
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Glu	Leu	Pro	Ala	Pro	Ala	Glu	Val	Ala	Ala	Lys	Asn	Gln	Ser	Leu	Glu
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	450					455					460				

Thr	Ser	Ser	Asn	Pro	Arg	Lys	Arg	His	Arg	Glu	Asp	Ser	Asp	Val	Glu	465		470		475		480
Met	Val	Glu	Asp	Asp	Ser	Arg	Lys	Glu	Met	Thr	Ala	Ala	Cys	Thr	Pro		485		490		495	
Arg	Arg	Arg	Ile	Ile	Asn	Leu	Thr	Ser	Val	Leu	Ser	Leu	Gln	Glu	Glu		500		505		510	
Ile	Asn	Glu	Gln	Gly	His	Glu	Val	Leu	Arg	Glu	Met	Leu	His	Asn	His		515		520		525	
Ser	Phe	Val	Gly	Cys	Val	Asn	Pro	Gln	Trp	Ala	Leu	Ala	Gln	His	Gln		530		535		540	
Thr	Lys	Leu	Tyr	Leu	Leu	Asn	Thr	Thr	Lys	Leu	Ser	Glu	Glu	Leu	Phe	545		550		555		560
Tyr	Gln	Ile	Leu	Ile	Tyr	Asp	Phe	Ala	Asn	Phe	Gly	Val	Leu	Arg	Leu		565		570		575	
Ser	Glu	Pro	Ala	Pro	Leu	Phe	Asp	Leu	Ala	Met	Leu	Ala	Leu	Asp	Ser		580		585		590	
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Leu	Leu	Ile	Asp	Asn	Tyr	Val	Pro	Pro	Leu	Glu	Gly	Leu	Pro	Ile	Phe		645		650		655	
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Gln	Tyr	Ile	Ser	Glu	Glu	Ser	Thr	Leu	Ser	Gly	Gln	Gln	Ser	Glu	Val		690		695		700	
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Tyr Lys Ala Leu Arg Ser His Ile Leu Pro Pro Lys His Phe Thr Glu  
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<213> Homo sapiens

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Leu Ser Leu Ser Thr Ala Val Lys Glu Leu Val Glu Asn Ser Leu Asp
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Ala Gly Ala Thr Asn Ile Asp Leu Lys Leu Lys Asp Tyr Gly Val Asp
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Leu Ile Glu Val Ser Asp Asn Gly Cys Gly Val Glu Glu Glu Asn Phe
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Glu Gly Leu Thr Leu Lys His His Thr Ser Lys Ile Gln Glu Phe Ala
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Ala Lys Val Gly Thr
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<210> 16

<213> Homo sapiens

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acttga						426